

DAIRY BACTERIOLOGY

BY

DR. ED. VON FREUDENREICH

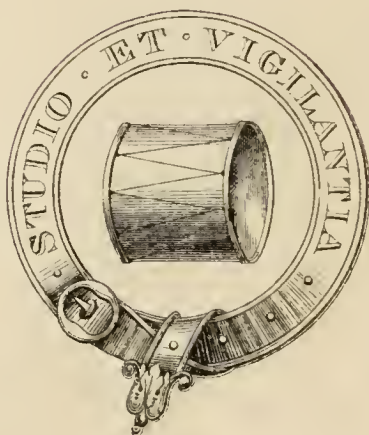
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J. R. AINSWORTH DAVIS B.A., F.C.P.

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DAIRY BACTERIOLOGY

A SHORT MANUAL

FOR THE USE OF

STUDENTS IN DAIRY SCHOOLS, CHEESE-MAKERS, AND FARMERS

BY

DR. ED. VON FREUDENREICH

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TRANSLATED FROM THE GERMAN

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AUTHOR'S PREFACE

This little book aims, primarily, at supplying dairy students with a brief treatise on bacteriology as applied to dairying. There are already many good books on the subject, as those of SCHOLL¹ and KRAMER² in German, and that of DUCLAUX³ in French. These, however, are intended more for students who themselves wish to work at bacteriology, being therefore somewhat too learned and exhaustive for those who mean to become cheesemakers or dairymen. For such students it is only necessary to get a general idea of bacteriology, and to become familiarized with the results so far attained by bacteriological research as regards dairying, and the practical application of the same. My own teaching of the subject in the Rütli agricultural

¹ H. SCHOLL,—Die Milch etc.,—Wiesbaden, 1891.

² E. KRAMER,—Die Bakteriologie etc.,—Vienne, 1890—92.

³ E. DUCLAUX,—Principes de laiterie,—Paris, 1893.

school is conducted in accordance with this principle. I have therefore introduced only so much of the general part of bacteriology, (culture and staining methods, morphology etc.), as is absolutely necessary for the comprehension of the special part (the bacteria of milk), and have made the whole as brief and elementary as possible. For this reason, as well as on account of its moderate price, it is hoped that this little work may find favour with established cheese-makers who have not, so far, had leisure to learn anything about bacteria, and with agriculturalists in general.

TRANSLATOR'S PREFACE

It is believed that the present translation of a popular work by a well-known authority will be of use to many who are more or less concerned with dairying. The great advance which Denmark has made during the last 30 years in the manufacture of butter is undoubtedly due, in great measure, to the enlightened way in which that country has seized every opportunity of applying science to practice. The uniformly good quality of Danish butter is largely due to the fact that the 'ripening' of the cream is properly regulated, pure cultures of bacteria being employed as 'starters', and assurance being made doubly sure by previously pasteurizing the milk to destroy noxious germs. Not only Denmark but America, France, Germany and Switzerland are far ahead of us in these matters, and compete against home dairy products with only too much success, while

Australia is rapidly becoming another serious rival. In 1892 the foreign butter imported into this country was valued at £11,965,190, the foreign milk (condensed or preserved) at £930,288, and the foreign cheese at £5,416,784. Rather more than half the butter came from Denmark and Sweden. Fortunately agricultural education in Britain is at last being properly organized, and the importance of bacteriology in dairying is gradually being realized.

The present work was first published in German ¹ during 1893, an improved French translation ² by the author following in 1894, during which year a still further improved Italian translation ³ was made by Dr. CARLO LA MARCA. The book has also been translated into Hungarian by Professor VINCENT VON NAGY. All the author's additions to the French and Italian editions have been taken advantage of by the present translator, and Dr. VON FREUDENREICH has been so good as to furnish further manuscript notes, so that the English translation is more complete than the other issues. A few trivial additions (marked Tr.) have been made by the translator.

¹ Die Bakteriologie in der Milchwirthschaft, CARL SALLMANN, Basel, 1893.

² Les Microbes et leur Rôle dans La Laiterie, CARRÉ, Paris, 1894.

³ I Microbi nel Latte e nella Lavorazione del Latte, CIOLFI, Cassino, 1895.

My best thanks are due to Dr. VON FREUDENREICH for his kindness in allowing me to translate the book, and for the help which he has given from time to time with the view of making it more generally useful.

J. R. AINSWORTH DAVIS

ABERYSTWYTH,

April, 1895

Among firms in this country supplying bacteriological apparatus Messrs. BECKER & Co., Hatton Wall, Hatton Garden, may be favourably mentioned.

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REVIEWS OF THE FRENCH EDITION

“As this book is so valuable, and as it is some time before it is likely to be—if ever it be—published in English, a short account of it may be readable to those at least who are interested in the subject.”—*The North British Agriculturalist.*

“To the dairy student, who can read French, we can heartily recommend this work, and hope that before long an English edition will appear.”—*The Dairy Review.*

TRANSLATED EXTRACT FROM REVIEW OF THE GERMAN EDITION.

“The appearance of this book will be welcomed by many farmers The book is of great value for cheese-makers, as well as for every agriculturalist concerned with dairying. For the investigation of faulty milk we regard it as absolutely indispensable.”—*Fühling's Landwirthschaftliche Zeitung.*

INTRODUCTION.

DURING recent years the theory of fermentation, of infectious diseases, and of similar phenomena has undergone very considerable modification. These appearances were at an earlier period regarded as being of a purely chemical nature (*Liebig's* fermentation theory, humoral theory, etc.), but the newer works of *Pasteur*, *Koch*, and others has proved that they result from the activity of the smallest forms of life, the forms known as micro-organisms. These two theories, apparently so opposite, have, however, been harmonized to some extent, for it has been shown that the action of micro-organisms depends in many cases on the production of chemical ferments known as diastases or enzymes. The modifications brought about by micro-organisms in dead or living matter are, therefore, immediately due to chemical action, which, however, is conditioned by the vital activity of the organisms in question. These new theories are of great importance

in dairying, for the fermentations and changes which milk and its products undergo—such for example as souring, a number of milk diseases, the ripening and certain diseases of cheese—result, as we shall see, from the action of various micro-organisms. This new field of study opened up only during the last few years has not yet been by any means perfectly explored, and many questions relating to it still need solution. The results so far obtained, however, are so important that it has become an absolute necessity for those concerned with the study and manufacture of dairy products to have at least some notion of this new branch of science, as in this way only is it possible to acquire the knowledge necessary for the rational treatment of these products.

Micro-organisms—under which name are included the smallest forms of life, which are not visible to the naked eye and can only be perceived when strongly magnified by means of the microscope—include numerous species. Their principal representatives are: **bacteria**¹ or **microbes**² with which we are chiefly concerned here; **yeasts**, which play a leading part in the fermentation of wine and beer; and **moulds**.

¹ Greek for *little staves*. Tr. ² Greek for *little beings*. Tr.

Before studying micro-organisms in detail, especially bacteria which affect the dairy industry, it is very desirable to give a brief general sketch of their structure and functions, as well as to learn the methods by means of which they are investigated.

HISTORICAL.

The chief group of micro-organisms, that of **Bacteria** or **Microbes**, is reckoned at the present time to include the smallest unicellular forms of microscopic plants. The credit of having first seen bacteria and of thus discovering a new world belongs to *Leuwenhoek*, a private individual who lived some 300 years ago at Delft (Holland), and by means of a simple microscope which had been constructed for his use examined all sorts of substances. To the astonishment of the scientific world of his time he demonstrated by means of this instrument that living organisms, of a kind up to that time quite unknown, were to be found in putrefying fluids, saliva, &c.

Moreover, he sketched these organisms so accurately that we can recognize them without hesitation as the bacteria of to-day. Since, however, no means were then known of isolating and

cultivating these objects, their nature remained quite uncertain, and they were commonly considered to be minute animals (animalcules). Scarcely any advance was made in our knowledge of bacteria until 1830, when the well-known naturalist *Ehrenberg* began a fresh study of them with the aid of improved optical instruments. His descriptions have even now some value, but the lack of culture methods prevented him from recognizing the true nature of these organisms, which he also classified with the Infusoria.¹ Some years later *Ferdinand Cohn* shed fresh light upon the subject. He showed, in fact, that bacteria are true plant cells, with which they agree in way of growth and division as well as in structure—and that they are connected with the higher family of algae² by a series of intermediate forms. It remained for *Pasteur* to make the greatest advance in this department by showing how to make pure cultures of these organisms, by which an accurate study of them was for the first time rendered possible.

¹ Microscopic animals first found in putrefying infusions of animal and vegetable matter. Tr.

² A large group of low plants including seaweeds, and the green, slimy and filamentous masses found in fresh water, etc. Tr.

But before considering bacteria in general and studying the results attained by the new methods, the question as to whence these organisms come has first to be answered. A prolonged controversy beginning in the 18th century took place in connection with this subject, and as will be immediately seen the scientific consequences of this have been of great importance. To put it briefly, those on one side regarded bacteria as produced from organic matter by the process of putrefaction, while those on the other side believed they were derived from living germs already present. The first theory is that of *abiogenesis*¹ or *spontaneous generation*; the second that of *biogenesis*² or "life from life." *Needham* (1747) was particularly prominent among the supporters of the former. Believing that all forms of life were destroyed by a simple boiling, he prepared a decoction of meat and as he found this putrefied later on, asserted that it gave rise to the bacteria which swarmed in it. *Spallanzani* (1777), on the other hand, showed that once boiling is not always sufficient to destroy all living germs, but that repeated and prolonged boiling, coupled

¹ From the Greek *a*, without; *biōs*, life; *gēnnaō*, I produce.

² From the Greek *biōs*, life; *gēnnaō*, I produce.

with exclusion of air and the microbes which it contains will entirely prevent meat broth, &c., from putrefying. In spite of this, however, the controversy was continued for a long time, until *Pasteur* demonstrated by methods which have since become classical, that all putrefaction is due to bacteria present in the air, and that blood, milk, urine, &c., will "keep" indefinitely if collected in such a way that they cannot be contaminated by germs, being then preserved in vessels free from germs, (*i.e. sterilized* vessels), and guarded against infection from the air. This theoretical discussion has practical bearings, for which reason it has been entered into somewhat fully. For if the theory of spontaneous generation were correct, it would be useless to fight against deleterious bacteria, as these would again and again be generated afresh. Fortunately, however, the truth is found in the contrary view, that bacteria only appear where their germs are already present, and it is sufficient to exclude these germs if their intrusion is to be prevented.

MORPHOLOGY¹ AND PHYSIOLOGY² OF
BACTERIA.

1. **Definition.**—We have already stated that bacteria are unicellular plants of microscopic size, *i.e.* not visible to the naked eye but only with the aid of the microscope. At such a low stage of life it is certainly difficult to draw a sharp boundary line between the animal and vegetable kingdoms, and it is not impossible that later investigators may include these organisms among animals, as was done by those who first observed them. Their plant nature is, however, generally admitted at the present time. In the vegetable kingdom they come nearest the algae, but for the most part possess no chlorophyll (leaf-green).

2. **Structure.**—Bacteria consist of an internal part, the *protoplasm*, surrounded by a skin or *membrane*. The latter consists of a substance related to cellulose,³ and swells up in some

¹ Morphology=the study of form and structure, (Greek, *mōrphē*, form; *lōgōs*, discourse). Tr.

² Physiology=the study of use or function (Gk. *phūsis*, nature, *lōgōs*). Tr.

³ Plant membranes are usually made up of this substance, a very good example of which is cotton-wool. Tr.

species to form a jelly-like capsule by which the plant-body is enclosed. It is not yet known with certainty whether a *nucleus*¹ is contained in the protoplasm, but there are many reasons for believing this to be the case. Such questions are not easy to solve owing to the minuteness of these objects.

3. **Shape.**—Bacteria may be of very different forms. Many are spherical, and these are termed *cocci* or *micrococci* (*sing.* coccus², micrococcus³) (Fig. 1, *a*). If such cocci instead of occurring singly are found in pairs they are termed *diplococci*⁴ (Fig. 1, *b*), while if arranged in chains they are known as *streptococci*⁵ (Fig. 1, *c*), and if in masses looking like bunches of grapes they are called *staphylococci*⁶ (Fig. 1, *d*). Bacteria may also be shaped like little rods (rodlets). If the rodlet is quite short it is spoken of as a *bacterium*⁷ (Fig. 1, *e*), if fairly long as a *bacillus*⁸ (Fig. 1, *f*). Very long filaments are included under the name of *leptothrix*.⁹ Bacteria are not

¹ *Nuclei* are particles of modified protoplasm which are stained very readily by means of dyes, Tr.

² Greek for *berry*. Tr. ³ Gk. for *little berry*. Tr.

⁴ Gk. for *double berries*. Tr. ⁵ Gk. for *chain berries*. Tr.

⁶ Gk. for *grape berries*. Tr. ⁷ Gk. for *little staff*. Tr.

⁸ Latin for *little rod*. Tr. ⁹ Gk. for *slender thread*. Tr.

in all cases straight, they may be curved or even corkscrew-shaped. In such cases (Fig. 1, *g*) we



Fig. 1. Micro-organisms, magnified to various extents.

speak of a *comma bacillus* (e.g. the comma bacillus

of cholera) or a *spirillum*.¹ Other kinds branch and are then distinguished by the name of *cladotrix*² (Fig. 1, *h*).

We must not, however, look upon these various forms as fixed and unalterable, constituting definite kinds or species. On the contrary, the same microbe may assume different shapes at different phases of its existence. We are acquainted, for example, with bacteria³ which, when young, appear in the micrococcus form, but later on elongate and become bacilli. Usually, however, one form predominates, so that within certain limits the differences in shape can be made use of for the purposes of classification, as will be seen immediately.

4. **Movement.**—Some bacteria are motionless, while others, on the contrary, execute spontaneous movements, and are provided with *flagella*⁴ (Fig. 1, *i*) which serve as organs of locomotion. Their movements may be extremely rapid or slow and sinuous.

¹ Latin for *little coil*, Tr. ² Gk for *branched thread*, Tr.

³ Throughout this book the term bacteria is used in the sense of microbes generally, but it may also be used as the plural of the special form *bacterium*. Tr.

⁴ Minute threads of protoplasm which execute lashing movements. (Latin for *little whips*). Tr.

5. **Size.**—As already stated, bacteria are so small that they cannot be seen with the naked eye.

Most bacteria are from $\frac{1}{25000}$ to several times that length of an inch long. It has been calculated that a thousand million of them could be packed into a hollow cube with edges $\frac{1}{25}$ of an inch long.

6. **Reproduction.**—Like all forms of life bacteria are able to reproduce. This is mostly effected by a process of splitting or *fission*, that is to say the microbe divides into two parts, each of which lives on and later on divides in its turn. If this process is watched under the microscope the coccus or bacillus will be seen to elongate somewhat and at the same time to get narrower and narrower until its two halves become free. On this account bacteria have also been called *splitting plants* (Schizophytes). In reference to the rapidity with which bacteria reproduce *Cohn* writes as follows :—

“Let us assume that a microbe divides into two within an hour, these two into four in the next hour, these again into eight in the third hour, and so on. The number of microbes thus produced in 24 hours would exceed $16\frac{1}{2}$ millions; in two days they would increase to 47 trillions, and in a week the number expressing them would be

made up of 51 figures. At the end of 24 hours the microbes descended from a single individual would occupy $\frac{1}{40}$ of a hollow cube with edges $\frac{1}{25}$ of an inch long, but at the end of the following day would fill a space of 27 cubic inches, and in less than five days their volume would equal that of the entire ocean."

According to *Cohn* again, a single bacillus weighs about 0.000,000,000,024,243,672 of a grain; 40 thousand millions, 1 grain; 289 billions, 1 pound. After 24 hours the descendants from a single bacillus would weigh $\frac{1}{2666}$ of a grain; after two days, over a pound; after three days, $16\frac{1}{2}$ million pounds.

It is clear that these calculations are merely hypothetical, and could only be realized if there were no obstacles to such rapid increase. Fortunately, however, lack of food and other checks prevent a multiplication of the kind. These figures give, notwithstanding, a striking illustration of the vital activity of these microscopic organisms. We shall also see later on how rapidly and to what a prodigious extent they can multiply in milk.

Some species, *e.g.* the different kinds of cladothrix, do not divide, but grow in length and give rise

to branched threads. Certain micrococci do not merely divide in one direction—a way by which diplococci and streptococci are produced—but in three directions simultaneously. In such cases bale-like masses are produced instead of simple rows. Cocci which grow in this way are included under the name *sarcina*.

A second and very important method of reproduction is by the formation of small bodies termed **spores**, which are able to resist unfavourable conditions. All bacteria, however, are not able to produce spores, but only certain species. Spores are of two kinds.

(a) **Internal spores** (Endogenous Spores). Under certain conditions in some kinds of bacillus the protoplasm is seen to contract into one or more refracting bodies. These are spores (Fig. 1, *j*). The membrane of the bacillus then breaks up and the liberated spores germinate later on into new bacilli.

(b) **Joint-spores** (Arthrospores). In this case the spore is not formed inside the bacillus, but an entire cell gets detached and becomes transformed into a spore, which is capable of germinating some time afterwards.

Spores have much more endurance and greater

powers of resistance than the bacteria from which they are derived.

Germination of Spores. A germinating spore becomes longer, and loses its bright appearance, after which the membrane surrounding it is ruptured and the young bacillus slips out. Certain conditions, such as warmth and the presence of oxygen, favour the formation of spores, while various kinds of bacteria secure their existence by developing spores when there is a lack of nutriment.

7. **Classification.**—The task of classifying bacteria is one of great difficulty, since they are comparatively little known and new kinds are constantly being discovered. It is possible, however, to arrange them in a few leading groups, according to their shape and the nature of their vital processes, such as spore-building and mode of growth.

The following classification, proposed by *Hueppe*, is one of the best:—

I. **Coccaceae.**—Forms which are cocci in the vegetative condition. They are subdivided into:

1. *Micrococci*;
2. *Sarcinae*;
3. *Streptococci*.

II. **Bacteriaceae.**—In the vegetative condition

these are rodlets, which under some circumstances may give rise to chains and threads. This group has the following subdivisions:

1. *Bacteria* (in the narrower sense), without internal spores or with joint-spores.

2. *Bacilli*, with internal spores.

3. *Spirobacteria* (comma-shaped and spiral forms).

III. **Leptothricheae**.—Rodlets and longish threads, which show a distinction between base and apex of the filament, growing out from a thinner base to a broader apex.

IV. **Cladothricheae**.—Forms consisting of branched filaments.

8. **Conditions of Life**. Like all other living beings bacteria are dependent upon certain external conditions:—

(a) They need, in the first place, a suitable **temperature**, which varies according to their kind, but in most cases must not be less than 59° F. nor more than 104° F., while from 86° F. to 95° F. is usually the most favourable. There are, nevertheless, bacteria which can multiply at 32° F., and others which can do so at 140° F. to 158° F. (the *thermophilous*¹ species closely studied by Globig and Miquel).

¹ Greek for *heat-loving*. Tr.

(b) **Oxygen.** In a large number of bacteria life is dependent upon the oxygen of the air—these are *aërobic* bacteria. On the other hand there are some kinds, *anaërobic* bacteria, to which free oxygen is a poison and which can only develop when it is absent. According as this relation to oxygen is absolute or relative, we speak of *obligate* aërobic and anaërobic bacteria, and *facultative* aërobic and anaërobic bacteria. Thus, for example, the bacteria of lock-jaw (tetanus) or of symptomatic anthrax can only flourish in the complete absence of oxygen, and are therefore obligate anaërobic forms. On the other hand bacteria such as those of anthrax are aërobic, but facultatively so, since they can live for a long time without oxygen.

(c) **Food.**—Bacteria, like other organisms, cannot live and reproduce unless they feed, and their food must contain compounds of carbon and of nitrogen. As they do not contain chlorophyll they are not able to extract the required carbon from carbonic acid gas (=carbon dioxide, CO_2), but are dependent upon carbonaceous compounds of more complex nature. They obtain their nitrogen from certain organic substances (albuminoids) as well as from some inorganic compounds (nitrates and compounds of ammonia).

9. **Saprophytes and Parasites. Pathogenic Bacteria.** Bacteria draw their nourishment either from dead substances or from living organisms. In the first case they are called **saprophytes**,¹ in the second case **parasites**. The latter prey upon some living organism or "host", and are termed *obligate* parasites if they can only live upon this host, *facultative* parasites if they can also live upon a dead substratum (*i.e.* upon dead matter). Saprophytes, on the other hand, live upon dead matter of organic nature, and are similarly divided into obligate and facultative saprophytes. **Pathogenic**² **bacteria**, *i.e.* those which give rise to various diseases, are classed with parasites, since they live and multiply in living organisms. They form one of the most interesting chapters of bacteriology on account of the part they play in producing diseases, especially those of infectious nature. All that was formerly known about such diseases was that they spread by means of some infectious matter, as, for example, in cholera, typhus, anthrax—but there was complete ignorance as to the nature of this matter. It would lead us too far to name all the diseases produced

¹ Derived from Greek words for *dead body* and *plant*. Tr.

² Greek for *disease-producing*. Tr.

by bacteria and to give in detail how the results have been attained. A single instance will serve to show how such conclusions have gradually been worked out. Farmers know and dread **anthrax**, an insidious disease which often attacks oxen and sheep, and is able to destroy entire herds in a short time. In 1849 *Pollender* examined microscopically the blood of animals infected with the disease, and observed minute rodlets in it. *Brauell* made a similar observation. *Davaine*, a French scientist, then showed, in 1863, that these rodlets were also the source of infection, since this blood when freed from rodlets by filtration through porous clay cells was no longer able to transmit the disease, while a drop of blood containing them produced anthrax when injected under the skin of an animal. Lastly *Pasteur* succeeded in propagating these bacteria outside the body—by means of culture methods to be described immediately—and in producing the disease by means of them in the same way as with the blood.

In a similar way bacteria have been proved to be the cause of a large number of other diseases, among which may be mentioned: malignant œdema, tuberculosis (consumption), leprosy, glanders, Asia-

tic cholera, typhus, pneumonia (inflammation of the lungs), diphtheria, erysipelas, suppuration, mammitis (inflammation of the udder), rabbit septicæmia, fowl cholera, erysipelas of swine, pleuropneumonia of swine, symptomatic anthrax, tetanus (lock-jaw), septicæmia of mice, actinomycosis, &c.

It has been proved that the pernicious action of these bacteria is especially due to the poisons (ptomaines and ferments) which they produce within the body. They may also be injurious for mechanical reasons, as when obstructions are produced by their excessive multiplication in the blood-vessels or when they abstract too much nutriment from the blood and various organs. These pathogenic bacteria which under ordinary conditions cause diseases and death can, however, have their properties so modified that they actually serve as a protection against these very diseases. The anthrax bacilli, for instance, if cultivated at a rather high temperature, are no longer fatal but only produce slight illness, which when withstood protects against virulent bacilli—in other words virus thus *attenuated* confers *immunity*. This discovery of the distinguished investigator *Pasteur* is at the root of the various kinds of inoculation which are destined to play a part of

ever-increasing importance in the conflict with infectious diseases. The methods now adopted for conferring immunity vary according to the disease (symptomatic anthrax, erysipelas of swine &c.), and space prevents a detailed description of them. It must suffice to have indicated the importance of these practical results.

The part which **saprophytes** play in nature is to bring about processes of fermentation and decay. They are of use to man because some of the fermentations they set up are of practical importance (lactic fermentation, "ripening" of cheese, &c.), and because as agents of putrefaction they get rid of dead bodies, converting them into simpler substances and thus bringing them again into the general circulation of nature.

10. **Various Life-Manifestations of Bacteria.**—Among the remaining life-manifestations of bacteria may further be mentioned:—production of colouring matters (pigments), of various gases and odours, and also in certain cases of light (phosphorescent bacteria). All these actions depend upon very complex chemical reactions which bacteria set up in the substances surrounding them by means of the ferments (diastases or enzymes) which they produce.

11. Resistance to External Influences.—

Like other living organisms bacteria are exposed to the action of external agents. Generally speaking their powers of resistance as regards these influences is tolerably high, especially when they are in the spore stage.

With reference to **heat** a distinction must be made between dry heat and moist heat. Spores are well able to withstand a dry heat of 266° F. for one hour. If any object is to be made free from germs, *i.e.* **sterilized**, it must be exposed to a temperature of 320° — 356° F. for half-an-hour, if this object is to be obtained with certainty. Damp heat has a more powerful action. Adult bacteria, without spores, are mostly killed at a temperature of 140° — 158° F. Many spores are killed by simply boiling, but there are, on the other hand, certain very resistant kinds which can support for a short time a temperature of 230° — 239° F. In order to sterilize culture fluids—the nature of which will be spoken about later on—it is necessary to expose them for about a quarter of an hour to a temperature of 239° F., a procedure which must of course be conducted under pressure in closed vessels of special construction.

Fractional sterilization is a method of ren-

dering culture fluids germ-free by exposing them to a temperature of not more than 140° — 158° F., for several times in succession, the operation extending over a number of days. By the first heating the adult bacteria are killed, the spores alone remaining alive; the liquid is then kept for 24 hours at a temperature favouring the germination of the spores, and then heated again. All the spores which have developed into bacteria are thus killed, and lest some of the spores should not have germinated the same procedure is followed for several successive days. This method is adopted for sterilizing liquids which are liable to alteration when exposed to very high temperatures. It has, however, been shown by *Miquel* that absolutely certain results are not attained in this way, for there are some spores which take days or even weeks to germinate. There is, therefore, the chance that such spores may be present and that they may ultimately germinate and people the liquids believed to be sterile.

Bacteria are extremely resistant as regards **cold**. Many bacteria, apparently in the spore form, are able to withstand an artificial cold of -202° F. for 20 hours. Bacteria, especially when in the spore condition, are mostly able to endure **desicca-**

tion for a tolerably long time. Certain kinds, however, *e.g.*, the cholera bacillus, are very sensitive in this respect and quickly die when they are dried up.

Light, especially direct sunlight, generally exerts a weakening action on bacteria. This supports the opinion of specialists in hygiene that the free access of light is desirable.

Bacteria easily resist a considerable **pressure**. *Certes*, for example, exposed anthrax cultures to a pressure of 600 atmospheres of compressed air without killing them. Again, *Dr. Schaffer* and myself have subjected various bacteria to a pressure of 90 atmospheres of compressed carbonic acid gas without any result.

As to the action of **electricity** upon bacteria, the results so far attained are very indefinite and more or less discordant. It is possible, however, that in the future this agent, the uses for which increase daily, may play a leading part in the destruction of bacteria.

Chemical reagents are destructive to bacteria in so far as they happen to be poisonous to protoplasm. This is why bacteria are killed by corrosive sublimate, carbolic acid, quicklime, copper sulphate, chlorine, &c. And on this account

these substances are frequently employed for purposes of disinfection.

THE METHODS OF BACTERIOLOGY.

Bacteria are chiefly studied by cultivating them outside the living organisms or dead substances which constitute their usual home. Only when this has been accomplished does it become possible to accurately investigate their structure and functions. One of the chief aims of bacteriology is, therefore, to devise means of making such cultures. At the present time this problem has been solved in the majority of cases, and just as the farmer sows grain or plants potatoes and later on harvests his crop, so the bacteriologist sows all sorts of bacteria in the fields of his artificial cultures, which afterwards yield a rich return. And just as the farmer first roots out the weeds from his field in order that the crop may not be impeded by them, so the bacteriologist begins by destroying all foreign germs in his culture fluids so that the bacteria introduced may develop freely. The composition of these nutritive substrata and culture fluids may vary considerably, but they must contain the substances

necessary for nourishing bacteria, more especially those of carbonaceous and nitrogenous nature. Meat broths, potatoes, plant decoctions, milk, &c., which contain such substances are, therefore, much used for the purpose.

Milk especially, which chiefly interests us here, is a rich culture fluid for bacteria. We may remember that its average percentage composition is as follows:—

Water 87.5

Dry substance consisting of:—

Fat	3.6	
Albuminoids	3.3	
(casein, albumin,		
and lactoprotein)		
Milk sugar	4.9	
Ash (salts)	0.7	12.5
		<hr/> 100.0

Casein, albumin, and lactoprotein, constituting the albuminoids (nitrogenous substances) are present as may be seen in sufficient quantity, while the bacteria can get their carbon from the milk-sugar and fat. Besides this, the constituents of the ash (compounds of potassium, sodium, calcium, magnesium, iron, phosphoric acid, sulphuric acid,

chlorine) also aid in the nutrition of the bacteria. All these substances exist in milk in a natural and consequently easily assimilable condition, and this is why milk is such a fertile soil for an enormous number of different sorts of bacteria. For the same reason it is much used as a culture fluid in bacteriological laboratories.

The various nutritious substances (milk, broth, &c.) are first placed in suitable glass vessels (test-tubes, flasks, &c.), and then sterilized in the way already described, so as to be rendered free from germs. These receptacles have their mouths stopped with plugs of sterilized cotton-wool, as a protection against infection from the bacteria always present in the air. The cotton-wool allows air to freely pass through it but keeps out all germs. If anaërobic bacteria are to be cultivated it is necessary to remove the air, or to replace it by some inert gas, such as hydrogen or nitrogen. The reaction of the culture fluid is not a matter of indifference, for too great alkalinity or too high a degree of acidity is fatal to bacteria. As a general rule, therefore, culture fluids are neutralized; but the reaction may be varied within certain limits according to the kind of bacteria to be cultivated. The nutritive media being

prepared they are sown, or technically speaking *inoculated*, with the microbe to be studied. Let us suppose that the anthrax bacillus is the one to be cultivated. By means of a platinum needle, previously sterilized by passing it through the flame of a Bunsen burner or spirit lamp, a minute drop of blood from an animal infected with anthrax, which blood is, therefore, known to contain the special bacillus, is introduced into a flask of broth. It is obvious that the procedure must be conducted with special precautions in order to prevent the culture fluid from being contaminated by foreign germs. Thus, the skin of the animal is disinfected with a 1 per 1000 solution of corrosive sublimate before the incision is made, and all the operations are conducted with perfectly sterile instruments. The broth is kept at a suitable temperature and the introduced bacilli multiply freely and constitute what is called a **pure culture**. It is now easy to study them by placing a drop of the culture under the microscope. To obtain pure cultures, however, is not always such an easy matter. We have often to deal with a mixture of various bacteria, as, for example, in fermenting and putrefying liquids, milk, &c., and it is necessary to separate these.

For this purpose solid culture media are used, prepared by addition of gelatine or agar (a Japanese seaweed) to culture fluids. In this way nutritive substances are procured which are jelly-like at temperatures suitable for the cultivation of bacteria (59° — 99° F.). Agar is employed for temperatures above 71° F., as gelatine liquefies under such conditions. If, for example, we wish to separate or isolate the different kinds of bacteria contained in a given specimen of water or milk, one of these gelatinous substances is liquefied by being heated in a test-tube to a temperature of about 113° F. (if a higher temperature is employed the introduced bacteria may be killed), and a drop or less of the liquid to be examined added. The mixture is then shaken up, so as to secure equal distribution of germs, and poured out upon a flat-bottomed receptacle previously sterilized. The gelatine (or agar) thus employed solidifies in cooling and constitutes a **plate culture**. If there is reason to believe that the fluid being investigated is very rich in germs it should be previously diluted with sterilized water. The bacteria enclosed in a plate-culture multiply at the spots where they are found to form **colonies**, which differ considerably accord-

ing to the nature of the microbes composing them. Some liquefy the gelatine, others do not; some are colourless, others, made up of pigment-forming bacteria, are coloured. By means of a sterilized platinum wire these colonies can be picked out and placed in separate receptacles containing suitable nutritive fluids, where they give rise to pure cultures of the different species which were present in the original fluid. In this way too, by counting the colonies present in a definite volume of gelatine it is possible to estimate the number of bacteria contained in a given liquid.

These various cultures must be kept at a temperature favourable to the growth of bacteria (see above), and for this purpose special boxes known as incubators are employed, the temperature of which can be kept at the required height (usually 68° F. and 95° F.) by special regulating apparatus.

When pure cultures have been obtained the bacteria composing them can be further studied, and are, to begin with, examined under the microscope. To study them in the living condition a small drop of the culture is placed upon a cover-glass,¹ which is then placed on a slightly

¹ A small square or circular piece of exceedingly thin glass,

hollowed glass slide, drop downwards. The bacteria remain suspended in a "hanging drop" culture of this kind, and it is possible to study their structure, observe their movements, &c. The details of structure are seen more distinctly in bacteria which have been killed and stained, advantage being taken in effecting the latter operation of the fact that the protoplasm of these organisms readily takes up certain dyes, especially those of aniline nature. A small drop of the culture is placed upon a cover-glass, dried by rapidly passing through the flame of a Bunsen burner, and then dipped in a staining solution. Bacteria can be seen much more distinctly in stained preparations of this sort than in the living condition. We cannot here treat in greater detail of these methods of preparation and staining, but they are of very great importance in bacteriology.

After this preliminary examination the pure culture is tested regarding its physiological properties (powers of producing fermentation, disease, &c.).

used for covering minute objects placed on a glass slip for microscopic examination. Tr.

HABITAT OF BACTERIA.

It follows from what has been already said that bacteria will be met with wherever organic life is found, and consequently wherever there are plants and animals, for the dead bodies of these furnish them with abundant food. They always abound, therefore, on the surface of the ground, and in water flowing in contact with the air. They are also often met with in large numbers in the air, into which they pass along with dust. The following figures will serve as illustrations.—

Earth.—Bacteria are very numerous in the superficial layers of soil, where they may occur to the number of hundreds of thousands per grain. They are less abundant in the deeper layers, and at a depth of from 10 to 20 feet the soil is generally free from germs.

Water.—Spring water issuing from deep within the earth is generally free from bacteria. The earth here acts as a complete filter, removing germs from the rainwater which passes through it and serves to feed springs. But after water has been exposed to the air it becomes charged with germs, as is the case with rivers, lakes, and the sea. Several hundred bacteria to $\frac{1}{16}$ of a

cubic inch is nothing unusual, and water containing something like this number of germs may nevertheless be fit to drink. But if the number is much greater than this there is reason for believing that the water is specially contaminated and for considering it suspicious.

Air.—The number of germs present in air is liable to great variation, depending as it does upon the presence or absence of sources of contamination. In uninhabited places there are generally less than 100 bacteria per cubic yard; in inhabited places several hundreds, a figure which may easily be raised to 200—400,000 per cubic yard if dust is stirred up, as, for example, by sweeping a room. In the streets of Paris there are on an average 4,000 bacteria per cubic yard—at Berne I have found about 700 in the same space. In open country fields I have found about 100 and on a small mountain (the Gurten, near Berne) not more than 8 per cubic yard. At a greater height, on the top of the Eiger (about 13,000 ft.), I have found air absolutely free from bacteria. The best way of calculating the number of germs in air is to pump a known quantity of it through a soluble filter, which is then dissolved in sterilized water and mixed with gelatine to

form a plate culture. The number of colonies which appears shows the number of germs present in the air examined. One of the best materials to use as a soluble filter is sulphate of soda, dried and placed in a glass tube through which air is drawn.

OTHER MICRO-ORGANISMS.

Before proceeding to study the bacteria of milk, the chief task here undertaken, a few words are necessary on certain other micro-organisms which though differing from bacteria play a similar part in nature.

1. **Yeasts.**—Yeast-plants or yeast fungi are micro-organisms of larger size than bacteria, generally of ovoid form and several thousandths of an inch in diameter. Their protoplasm is granular and contains vacuoles, *i.e.* spaces full of fluid. They do not, like bacteria, multiply by division (fission), but by budding or sprouting, that is to say they give rise to little buds or sprouts which gradually grow larger and ultimately break away from the mother cell (Fig. 1, *k*). Sometimes, especially when growing upon solid substances, they grow into slender thread-like structures (constituting a “mycelium”, as in

the moulds spoken of below), and may produce spore-cases (sporangia) in which spores are developed. The yeast fungi play an important part as the agents by which the fermentation of wine and beer is produced.

2. **Moulds.**—The whitish and variously coloured moulds which grow upon old bread, jam, &c., are familiar to everyone. They consist of minute fungi, which on account of their small size may be classified as micro-organisms, and have some affinity with bacteria. They belong to the subdivision of Cryptogams (plants which do not produce seeds) and among these to the extensive group of Thallophytes, which includes plants that are not divided into stem and leaves but possess a simple plant-body known as a thallus. In moulds the thallus is made up of cells devoid of chlorophyll but consisting, as in bacteria, of a membrane and protoplasm. They do not propagate by splitting (fission) but by growth in length, leading to the production of elongated threads termed **hyphae**. These branch and become entangled together in a thick feltwork known as a **mycelium**. Some of these hyphae are fertile and bear fruit or spores, also known as conidia. A very large number of genera and species of

mould are known, among which the following may be mentioned.

(a) **Penicillium** (fig. 1, *l*) receives its name (Latin for *painter's brush*) from the shape of its fertile hyphae, which fork repeatedly so as to end in a tuft of short stalks (basidia) at the tips of which chains of spores are developed. One species, *Penicillium glaucum*, a familiar green mould, is found for example on Roquefort cheese, in the maturing of which it plays an important part. The green vein-like markings seen in this kind of cheese consist of masses of the mould.

(b) **Aspergillus** (fig. 1, *m*). Blue Mould. In this case the ends of the unbranched fertile hyphae are swollen up like a club, and from each of them a number of small flask-shaped structures (sterigmata) project, upon which the spores are borne.

(c) **Mucor** (fig. 1, *n*). White mould.—Each of the fertile hyphae ends in a rounded spore-case or sporangium (*sp.*). When this is ripe it bursts and liberates the spores. (II=magnified sporangium with spores.

(d) **Oidium** (fig. 1, *o*). This mould develops a mycelium, the hyphae of which break up into short joints. *Oidium lactis* is frequently met with in milk.

THE BACTERIA OF MILK AND THEIR PROPAGATION.

IT follows from what has already been stated that bacteria find milk to be an excellent nutritive substratum, since it contains in the most suitable form all the food-stuffs which they require. It is therefore not astonishing that when a drop of milk is submitted to examination it is usually found to contain a large number of bacteria. When in the udder, however, milk is free from bacteria, except when the milk glands are in a diseased condition, as the result, for example, of tuberculosis or of inflammation. In such cases the milk as it leaves the udder will contain tubercle bacilli or the bacteria which set up inflammation. But these cases excepted, it is, to begin with, free from germs, a fact demonstrated by *Pasteur*, and one the truth of which can easily be made evident by drawing milk direct from the udder with the aid of a sterilized cannula (small tube). If, on the other

hand, milk obtained in the ordinary way is submitted to examination it will be found as a regular thing to contain very numerous bacteria. In Berne I have found on an average 160—320,000 bacteria per cubic inch in fresh milk, while *Cnopf* in Munich estimates the number at 960,000 to 1,600,000 per cubic inch, *i.e.*, 33 to 56 millions per pint! Whence come these bacteria? the answer is easy if the conditions under which milking takes place are carefully considered. To begin with, the bellies and udders of the cows are soiled with dung, which is well known to harbour innumerable bacteria, and fragments of which fall into the milk. Again, the hands of the milker are usually not too clean and therefore introduce numerous germs, while the milk pails or cans are in many cases washed with water rich in bacteria, and the air of the cowhouse contains a large number of them. To this must be added that the first half gill or so of milk obtained is always rich in micro-organisms, since after milking a little milk remains in the lower part of the teat, where it is not completely shut off from the exterior and is in consequence easily infected, so that a rich crop of bacteria is produced in it by the next milking-time. How much this helps to con-

taminate milk has been shown by the observations of *Dr. Schultz*, from which it appears that the first-drawn milk contains some 1,360,000 germs per cubic inch while the last is sterile, *i.e.*, free from bacteria. The importance of clean hands is shown by an experiment in which, following a recommendation of *Professor Guillebeau* of Berne, the hands of the milker were greased so as to prevent impurities from rubbing off. In this experiment the milk was drawn into a sterilized receptacle, and the number of germs was diminished to 3,400 per cubic inch. Since, as will be seen immediately, it is bacteria which make it difficult to preserve milk, greater cleanliness and care in milking would result in the production of an article with much improved keeping properties. With this object in view the hands of the milker and the cows' udders may with advantage be washed in lukewarm water, while the vessels for holding the milk should be kept as clean as possible, and greater cleanliness be maintained in the cowhouse.

Let us now see what comes of these bacteria which are regularly present in milk. As they find in this liquid an abundance of excellent food it is clear that they will multiply freely, and the

more favourable the temperature the more rapidly will this take place. Some instances taken from experiments conducted by me here in Berne, will make this clear.

A sample of milk containing 153,000 bacteria per cubic inch was kept at a temperature of 59° F.

1 hr.	after it contained	539,750	bacteria per cu. inch.
2 hrs.	„ „ „	616,250	
4 „	„ „ „	680,000	
7 „	„ „ „	1,020,000	
9 „	„ „ „	2,040,000	
25 „	„ „ „	85,000,000	

Samples of another milk, containing a short time after it was taken from the cow 391,000 bacteria per cubic inch, were kept at temperatures of 77° F. and 95° F. The following table shows the rate of increase of bacteria in the two cases.

at 77° F.		at 95° F.	
2 hrs.	after (plate liquefied at time of examn).	1,275,000	bacteria per cu. inch.
6 „	„ 14,620,000	45,900,000	
9 „	„ 36,550,000	57,800,000	
24 „	„ 13,702,000,000	13,812,500,000	

These figures, to which if space allowed I could add many others, sufficiently prove the influence of temperature. The lesson may be drawn from them that new milk must be kept as *cool* as possible,

so as to prevent a too rapid increase of bacteria, since, as will presently be seen, they are the chief cause of the changes which take place in milk.

NATURE AND ACTION OF THE BACTERIA FOUND IN MILK.

We have noted the presence of bacteria in milk as well as facts relating to their multiplication, and it now remains to consider what these bacteria are and what part they play.

The bacteria met with in milk can be classed in two chief groups.

There are, in the first place, those which may be regarded as the constant or at least very frequent inhabitants of milk. Just as there are animals which are only found in certain regions or particular climates, so are there bacteria which in the course of centuries have become so accustomed to live in milk that they are almost constantly met with in the various dairy products. These kinds are naturally of most interest in dairying, as it is they which are the cause of most of the alterations taking place in milk.

There are, on the other hand, a large number of bacteria which though frequently met with in milk can only be considered as accidental guests.

These are of less importance in dairying. There is always, however, the possibility of such kinds getting into milk and, finding it a congenial soil, undergoing multiplication with production of poisonous substances. Cases are therefore known, especially in America, where cream, cheese, &c., have led to poisoning. It is therefore well to shield milk from the action of *all* bacteria. When the casually occurring bacteria belong to the pathogenic (disease-producing) group of microbes they have a very special interest both for producer and consumer, as in such cases the milk is a means of spreading various diseases. Bacteria of this kind are so important that they may well serve as the subject of a special section.

I. PATHOGENIC MILK BACTERIA.

1. **Bacillus of tuberculosis.**—It is but rarely that tubercle bacilli get into the milk after it has been drawn from the udder, but this may happen, as, for instance, when it is contaminated by the expectorations from diseased lungs or by other matters containing the bacilli. When this bacillus is met with in milk it is generally owing to tubercular disease of the milk-glands so that infection takes place before the milk leaves the

udder. It is, however, also found in cases of cows affected with tuberculosis, where, notwithstanding, the udder appears to be sound. As to its frequency in milk, accounts differ according as the observations have been made in regions where tuberculosis (consumption of cattle) is more or less common. According to *Hirschberger*, for example, 10 per cent of the cows living in the neighbourhood of towns, where they are not generally treated in a very rational way, suffer from tuberculosis, and 50 per cent of these yield milk containing tubercle bacilli. According to this 5 per cent of the samples of town milk contain these microbes. That the bacilli so found are *virulent*, *i.e.*, able to cause disease, has been proved by subjecting suitable domestic animals to hypodermic inoculation (injection of infected milk under the skin), and by feeding such animals with infected milk. In both cases the animals experimented upon contracted the disease. It must be remembered that in the case of tuberculosis, as with other infectious diseases, the number of bacilli introduced into the body is of importance, for infection takes place more readily when this is large. In practice, the chances of infection are consequently diminished by the fact that milk from a number of cows is

generally mixed together before sale in the market, so that any infected samples are diluted. This dilution, however, as recent experiments have shown, only *reduces* the risk of infection, and does not do away with it altogether. In Copenhagen, for example, 4 out of 28 samples of mixed milk, *i.e.*, $\frac{1}{7}$ of them, proved virulent when injected under the skin. Two of these samples came from dairies where from 20 to 30 cows were kept, and in each case there was only one cow suspected of being tuberculous. The remaining two of the infected samples were more virulent, and these came from dairies where there were not only suspected cows but also cases where the udders were visibly tuberculous. Although tuberculous milk when consumed does not necessarily cause disease, since a certain predisposition, or weakness of the digestive organs, may be necessary for infection, or the bacteria may not be present in large enough numbers, such milk is, nevertheless, a grave source of danger to health, and many instances can be quoted where the appearance of consumption in children is traceable to no other cause. Thus *Brouardel* cites a case where 5 out of 14 young girls living together in a boarding-house became consumptive, subsequent to the

daily use in the establishment of milk from a tuberculous cow.

The tubercle bacilli can remain alive for a long time in dairy product. *Gasperini*, after adding some of them to potted butter, found them still living after 120 days, while *Galtier* under similar circumstances found them still living in cheese after a lapse of 35 days. After a month, however, they appear to be attenuated, so that their presence in cheese does not seem to be a great source of danger, especially as this kind of dairy produce is not usually consumed till several months after it has been made. The facts that have been mentioned justify the advice given by authorities on health to boil milk before using it as food, with the object of destroying any tubercle bacilli that may happen to be present. The best plan, however, would be never to employ tuberculous cows for the production of milk.

2. **Typhus.**—As far back as 1870 a local epidemic of typhus, which broke out at Islington, was traced with tolerable certainty to the use of a particular sort of milk. In fact the only persons who contracted the disease were those supplied with milk from a dairy where a case of typhus had previously appeared. Since this many cases

of a similar connection have been proved. Thus *Jaccoud*, in France, cites 106 cases of typhus, in 17 of which milk was the means of infection, and *Hart*, in England, has proved that out of 50 typhus epidemics 28 were due to infected milk. It is not difficult to explain how, in such a case, typhus germs get into the milk. Excrementitious matters are often able to filter through from dry closets into wells or brooks, and if there are cases of typhus in the house the water is then contaminated with typhus bacilli, large numbers of which are found in the dejecta of persons suffering from the disease. This leads to infection of the milk if the milk-cans, &c., are washed in the contaminated water. A more direct transmission becomes possible if the persons who nurse the sick also help in the dairy. As to the length of time for which the typhus bacillus can live in dairy products, *Laser* has proved that this may be 5 to 7 days in the case of butter.

3. **Cholera.**—The bacillus of cholera, like that of typhus, is able to contaminate milk. In boiled milk this micro-organism is able to multiply abundantly. It appears, on the contrary, according to the researches of *Cunningham*, that in unboiled milk it is overpowered within 24 hours, by the

ordinary milk bacteria, being killed by the acid produced. A case is known, however, where all the sailors on board a ship sickened with cholera, after drinking milk which had been mixed with water contaminated by choleraic dejecta. It has also been shown by *Laser* that cholera bacilli can live in butter for 4 or 5 days.

In the case of cheese the cholera bacilli do not appear to be a source of danger. In fact, *Weigmann* inoculated skim milk with cholera bacilli in such a way that the proportion between their number and that of the ordinary milk bacteria present was 3 to 5. The milk was then used to make small Limburg cheeses, in which none of the cholera bacilli could be found alive after a lapse of 22 hours, except in a single instance. As such cheeses are not sold till a much longer time than this has elapsed, it is impossible for them to propagate cholera. Nor is danger to be apprehended from cheeses which are eaten when new, for these are made from sour milk, in which the cholera bacilli quickly die. *Heim* inoculated $1\frac{3}{4}$ ounces of curd with an entire colony of cholera bacilli grown upon agar, and after 26 hours could not find a single living bacillus.

4. **Other pathogenic bacteria.** --Diphtheria

and scarlet fever seem to have been spread by infected milk in a number of cases. According to *Dr. Hart* 14 epidemics of scarlet fever and 7 of diphtheria have been traced to this source in England. The microbe of scarlet fever has, however, not yet been recognized, so that in this case there has not been strict proof, though the view taken may be regarded as probable. Again, many cases can be quoted where the use of a particular kind of milk has been followed by intestinal derangements undoubtedly due to the action of special bacteria. Diseases of animals, such as pleuropneumonia, foot and mouth disease, &c., can also be spread by means of milk. In any case the facts which have been adduced prove sufficiently that milk may be the means of transmitting numerous diseases when it contains their germs. This question is of importance, not only in reference to the public health, but also on account of the injury it may inflict on dairy associations. If, for example, the milk of a particular dairy were proved to disseminate typhus the result would certainly be to stop the sale till the source of contamination were found and removed. This would often take a considerable time, and the consequent interference with

the business of the dairy would involve large pecuniary loss for those interested. Too much care cannot then be taken in order to prevent the infection of milk by pathogenic bacteria.

II. ORDINARY MILK BACTERIA.

We now pass on to bacteria of which milk is the usual habitat, and which can therefore be considered as true or normal milk bacteria. It is these which possess a special interest for us since they bring about numerous alterations and decompositions in milk, of which some are useful and others harmful for dairying purposes. Thus, for example, certain dairy products, such as cheese, soured milk, ripened cream, and other things, are the result of fermentative processes caused by bacteria. Such microbes should, therefore, be the objects of our care and protection, as in the case of yeasts to which we owe the fermentation of wine and beer.

The ordinary bacteria of milk are classified according to the effects which they produce in milk. The following groups may be specially mentioned.

1. **Lactic Ferments.**—If some fresh milk is left to stand, it will be found curdled after from 2 to 4 days, and will at the same time have

acquired an acid reaction. This curdling is the work of lactic ferments or bacteria. There are, in fact, not one species only but quite a number of bacteria which possess the power of setting up fermentation of milk-sugar, *i.e.*, of causing it to split up into carbonic acid and lactic acid. It is well known that a certain degree of acidity in milk makes it curdle, as when, for example, acetic acid or lactic acid is added to it. In consequence of this the lactic acid formed in milk by the action of bacteria causes curdling as soon as enough of it is produced. Lactic bacteria are classified as *specific*, the usual cause of the souring of milk, and *facultative*, *i.e.*, those which are not usually found in sour milk but can set up lactic fermentation when sterile milk is inoculated with them. The latter are, therefore, not to be reckoned as true lactic bacteria, but belong to the group of those bacteria which get into milk accidentally. They consequently possess no further practical interest for us and need not be considered any more.

Among the chief representatives of lactic bacteria the *lactic bacillus* of Hueppe is generally mentioned, which was made the subject of a careful investigation by this scientist. In shape this bacillus is a

short thick rodlet of from $\frac{1}{25000}$ of an inch long to $1\frac{3}{4}$ that length, and $\frac{1}{62500}$ of an inch thick or rather less. It grows at temperatures between 50°F . and 113°F ., most rapidly at 95°F . Introduced into sterile milk it produces uniform curdling within 15 hours, at a temperature of 77°F . to 86°F ., as a consequence of which the milk loses its fluid character. The curdling produced by its action is not followed by liquefaction. Indeed, it may be stated as a general rule for normal lactic bacteria that they do not possess the power of liquefying curdled milk, and rarely liquefy gelatine. It is true that in practice curdled milk often becomes fluid again, acquiring at the same time an unpleasant smell and taste, but this is the work of other bacteria which develop later and which will be spoken of in the sequel.

Among lactic bacteria we may further mention the *lactic bacillus* of *Grotenfelt*, which produces alcohol as well as lactic acid and carbonic acid; *Grotenfelt's* lactic bacterium,¹ *Hueppe's* 1st and 2nd lactic micrococcus,² *Marpmann's* lactic micrococcus, lactic sphaerococcus, and lactic bacterium,³

¹ *Bacterium acidi lactici*, Grotenfelt. ² *Micrococcus lactis*, I and II, Hueppe. ³ *Micrococcus acidi lactici*, *Sphaerococcus acidi lactici*, and *Bacterium limbatum acidi lactici*, Marpmann.

Krueger's lactic micrococcus,¹ which liquefies gelatine, and *Grotenfelt's* lactic streptococcus.² This by no means exhausts the list of lactic bacteria, and new kinds will certainly yet be discovered. In Emmenthal cheese, for example, I have discovered lactic bacilli and micrococci which have not yet been described.

Lactic bacteria do not as a rule develop spores, and are not, therefore, very resistant to heat, a temperature of about 158° F. generally killing them. These species are also troublesome because they are one of the chief difficulties in the way of preserving milk, since they are the cause of spontaneous curdling; or even if they have not brought this about they may have made the milk so acid that it will not stand boiling without the formation of light flocks, an occurrence only too well known to all housewives. Sometimes, on the other hand, these lactic bacteria are useful, as in cases where milk needs to be acid to some extent before it is used. Thus, in France and Germany almost all butter is made from slightly sour cream, as is also the case with Swiss butter intended for exportation (especially to Paris), since

¹ *Micrococcus acidi lactici*, Krueger. ² *Streptococcus acidi lactici*, Grotenfelt.

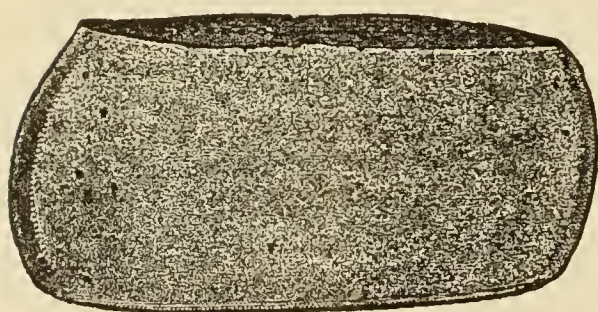
it keeps much longer and suits better the taste of the consumers. The method is well known. A small quantity of slightly sour milk is added to the cream, which is then allowed to stand till the following day. The cream is then slightly sour and ready for churning. In order to have every day the proper quantity of sour milk in the right state to act as a 'starter,' it is necessary each time to add from 2 to 4 pints of it to fresh or, still better, pasteurized milk or skim milk (pasteurization will be treated of later). This milk will be just in the right state to add to the cream on the following day. In order that the butter should be good this ripening of the cream must be perfectly normal, and since bacteria cause it everything depends upon their nature. If, for example, the milk added to the cream in order to ripen it should happen to contain some of the bacteria which produce evil-smelling substances in milk, the cream will acquire a disagreeable odour and a bad taste. It is, therefore, necessary to take care that the milk employed for this particular purpose contains only bacteria which produce proper ripening and an agreeable aroma. *Weigmann* of Kiel has succeeded in finding such a microbe, and by ripening cream

with pure cultures of it has prepared butter of excellent quality. The method employed is the same as before, except that the cream is ripened with milk rendered acid by sowing a pure culture in it instead of with milk which has turned sour of itself. According to *Weigmann* it is best to pasteurize the milk (or skim-milk) used, as the bacteria added might otherwise be overpowered by other kinds already present. By thus providing in each occasion for a supply of sour milk with which to inoculate the cream of the following day it is possible to obtain a product of uniformly good quality. *Weigmann's* microbe (a micrococcus), however, is not the only species capable of setting up normal lactic fermentation in cream, and others are sure to be discovered which will suit still better the tastes of different countries and towns. We have an instance here, therefore, of a useful part played by bacteria in dairying, and of the way in which they can be employed for this end.

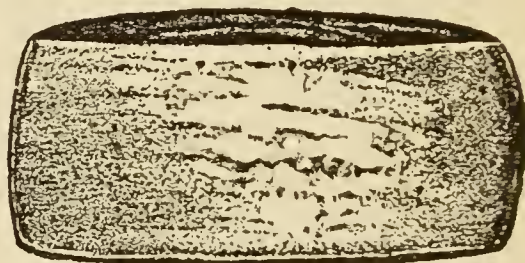
Unfortunately the contrary is often true, for there are other lactic bacteria which are able to exert a harmful action, especially as regards the manufacture of cheese. The kinds referred to are those which split up milk-sugar with so much

energy that gas is produced with great rapidity. If, for example, a decoction of sugar is inoculated with one of these bacteria and kept at a temperature of 95° F. bubbles will already be seen to rise in 6 or 7 hours, especially when the flask is shaken. It is easy to understand that when large numbers of such bacteria are present in cheese the gases produced will cause it to 'heave' and lead to the production of abnormally large holes.

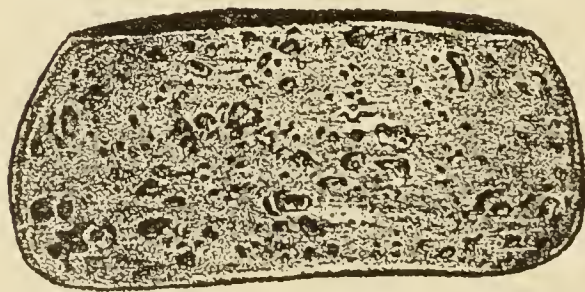
Figure 2 makes this clear. Here one of the

*a*

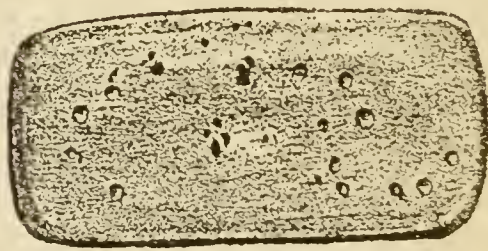
a. Cheese prepared from milk previously inoculated with one of the bacteria which cause heaving. Three days old.

*b*

b. Control cheese.



Section through *a*.



Section through *b*.

Fig. 2.

trial-cheeses, made from 17 pints of milk is still normal; the second, to the curd of which a culture

of these bacteria was added, is considerably swollen after only three days. This group of bacteria undoubtedly includes a large number of forms, of which only a small part have as yet been carefully studied. In Switzerland I have most frequently found in cavernous cheeses the micro-organism to which I have given the name *Schaffer's* bacillus.¹ I also found it in the cheeses called "thousand holed," and by means of cultures of it produced this porous appearance as well as the cavernous structure alluded to. In fact I observed that when the bacteria are scattered here and there in heaps large cavities are produced like those of cavernous cheese, while when they are very numerous and evenly distributed, innumerable small holes are produced at almost every part of the cheese. These holes are so near together that they cannot become bigger. This is a similar thing to what is seen when gelatine plates are sown with too many germs, so that the colonies remain very small, while in plates less thickly sown they are able to extend. This bacillus appears to be very widely distributed and I have found it among other places upon potatoes. It

¹ *Bacillus Schafferii*. Landw. Jahrbuch der Schweiz, 1890, II, p. 17.

is, therefore, better to avoid the widely-spread continental practice of smearing the hands with potato broth before milking, for in this manner the unbidden guest may be brought into the milk. *Dr. Schaffer*, the cantonal chemist at Berne, has already in addressing popular meetings spoken against this dirty habit, and I have therefore given his name to the bacillus. The potato broth may with advantage be replaced by lard.¹

Schaffer's bacillus is undoubtedly related to a kind of bacterium (*Bacterium coli commune*) always found in the intestine, and it is therefore advisable to take the greatest care lest the milk should be contaminated by cow-dung during milking.

Certain other gas-producing lactic bacteria are also responsible for inflammation of the udder, and this is of interest in reference to dairying since milk from cows thus affected is not fit for cheese-making, owing to the presence of these bacteria. If, therefore, cheeses begin to develop a cavernous structure it is necessary to have the cows from which the milk is obtained carefully examined by a veterinary surgeon. Among such bacteria are to be reckoned three sorts of bacillus

¹ Compare p. 38.

(*Bacillus Guillebeau*, a, b, and c.) found by *Prof. Guillebeau* in cases of inflamed udder, and which I have proved to possess the power of causing well-marked cavernous structure in cheeses inoculated with them.

We must also add to the list two species of micrococcus discovered by *Adametz* at Sornthal, where they produced cavernous structure in cheese and were also considered by him to cause inflamed udder. Several kinds of micrococcus and streptococcus (*Adametz*, *Macé*, *Hueppe*) met with in cases of the same complaint must further be mentioned. The experimental proof of their power to produce cavernous structure in cheese is not yet complete, but this is probably the case, for when sown in milk they generally set up active fermentation.

This, however, by no means completes the list of bacteria which can cause cavernous structure in cheese. Besides those already mentioned as possessing the power of splitting up milk-sugar into lactic acid and carbonic acid, there are a series of bacteria which by the decompositions which they set up give rise to large quantities of other gases, for example, hydrogen. If this production is very energetic cavernous structure

will result, for which reason these bacteria are mentioned here though they do not belong to the lactic ferments. Thus, for example, *Weigmann* discovered and studied two kinds of milk bacillus which he found produced holes as large as pigeons' eggs in small trial cheeses. To these must be added a bacillus (*Bacillus actinobacter polymorphus*) described by *Duclaux*, and other bacilli which the same observer found in soft cheeses.

Lastly, certain yeasts are able to set up fermentation in curd, but this probably concerns soft cheese only, as hard cheese would not be a good soil for them. Later on we shall study some of these yeasts.

2. Ferments of Casein.—The members of this second and very numerous group of bacteria are also able to curdle milk, not, however, by producing lactic acid but by formation of a rennet-like ferment. When these bacteria are cultivated in broth, for example, and this is afterwards freed from germs by being passed through a porcelain filter it curdles milk as rennet does. One microbe of this kind (*Tyrothrix tenuis*) cultivated by *Duclaux* in milk, at a temperature of $98\frac{1}{2}^{\circ}$ F., was able to curdle thirty times its own weight of

milk in 11 minutes, and 120 times its own weight in 2 hours. *Conn* also succeeded in preparing from cultures of another species of the sort a ferment, in the form of a powder, which acts like rennet. These bacteria also possess the power of redissolving, by means of a second ferment, the milk which they have curdled. This ferment has been called *casease* by *Duclaux*, and it probably plays an important part in the ripening of cheese, since the characteristic changes which the casein undergoes during this process are to be ascribed to its action. These changes are not limited to mere curdling with subsequent dissolving, but involve complex decompositions resulting in the production of various substances, such as peptone, leucin, tyrosin, ammonia, butyric acid, &c.¹ Butyric acid is very frequently met with in these fermentations, and a special group of *butyric bacteria* has even been constituted. Such a division, however, is scarcely justifiable, for butyric fermentation is not an uniform process. Butyric acid is rather to be regarded as a residue resulting from the breaking-down of casein and milk-sugar in various ways; it is only, in fact, like leucin, tyrosin, and

¹All but the last of these are more or less complex nitrogenous compounds. Tr.

ammonia, the final product of diverse kinds of fermentation. It is, therefore, better, in accordance with the suggestion of *Duclaux*, to place all these various microbes in a common group of *casein ferments*. They are for the most part spore-forming bacilli, and often possess almost incredible powers of resistance. Thus, for example, according to *Duclaux*, the above-mentioned *Tyrothrix tenuis* can stand for about a minute a moist heat of from 239° F. to 248° F. It is more especially these bacteria which make it so difficult to completely sterilize milk, as will be seen later. It is quite impossible to describe here all the kinds which make up this group, for there are an immense number of them, most of which have not yet been studied. The best known are those found by *Duclaux* in Cantal cheese, to which he has given the name of *Tyrothrix*,¹ and the action of which on milk he has carefully studied. Then come many of the innumerable host of *hay* and *potato-bacilli*, so called because first found in hay infusions and on imperfectly sterilized slices of potato, but which are no other than some of the widely-distributed microbes that everywhere live

¹ *Tyrothrix tenuis*, *filiformis*, *distortus*, *geniculatus*, *turgidus*, *scaber*, *virgula*, *urocephalum*, *claviformis*, *catenula*.

on the surface of the soil. Lastly may be mentioned the bacilli described as butyric ferments by various authors.

It has already been stated that according to *Duclaux*, these bacteria probably play a leading part in the ripening of cheese. That this is actually the case may now be regarded as proved by numerous researches which have been conducted on the subject. Thus *Dr. Schaffer* and *Dr. Bondzynski* showed some years ago that cheese made from boiled milk, and therefore free from bacteria, will not ripen. *Adametz* has demonstrated the same thing for cheese to which bacteria-killing substances, such as creolin and thymol have been added; and, lastly, a long series of experiments conducted by myself prove that ripening hardly ever takes place when the milk from which cheese is to be made is pasteurized, so that most of the bacteria present are destroyed (*see later on*). From this it undoubtedly follows that without bacteria there can be no ripening. But it is still an open question whether this ripening process simply depends on the above-mentioned casein ferments, or upon special cheese-ripening bacteria at present unknown and unstudied. In favour of the former view the fact may be men-

tioned that pure cultures of the bacteria classed as casein ferments contain the same products as those formed in cheese during its ripening (leucin, tyrosin, &c.); in support of the latter view it may be stated that so far, at any rate, we have not succeeded in hastening or improving ripening by adding cultures of such bacteria to cheese. Further experiments, which I have just made for the purpose of clearing up this point have made me believe that the part which these so-called casein ferments play in the ripening of cheese is more than doubtful. I have shown that when fresh cheese is inoculated with large numbers of them they disappear very rapidly, and in fact do not multiply at all. I may add that I agree in this point with the ideas set forth by F. S. Lloyd, F.C.S., F.I.C., in his most valuable papers on the manufacture of Cheddar cheese (Reports for 1893, Bath 1893). The ripening of cheese is apparently a very complex matter, in which several factors co-operate.

Lactic bacteria are always very numerous in cheese, and I believe play a sufficiently important part to constitute a new field of study.

3. **Blue Milk.**—A common disease of milk consists in the appearance of blue patches which

may remain limited to certain spots or may extend over the whole surface. According to the temperature this condition is produced in from 24 to 72 hours. *Steinhoff* so long ago as 1838, and *Fuchs* in 1841, showed that this disease of milk is infectious, while the latter observer proved the presence in such milk of a special microbe, which, however, he did not succeed in cultivating because the methods of bacteriology were too imperfect at that time.

This microbe, the **blue milk bacillus** (*Bacillus cyanogenus*), was afterwards specially studied by *Neelsen*, *Hueppe*, *Heim* and *Gessard*. It is from $\frac{1}{25000}$ to $\frac{1}{6250}$ of an inch long and $\frac{1}{83000}$ to $\frac{1}{50000}$ of an inch thick, and performs very active movements. It does not liquefy gelatine, but gives it a dark colour. The production of a blue colour in milk by means of this microbe is dependent upon certain conditions, which furnish a beautiful example of what is known as **mutualism** (symbiosis ¹) of bacteria, *i.e.*, the production in certain cases, of special effects entirely dependent on

¹ Mutualism is also illustrated by:—1 The swellings or 'tubercles' in the roots of leguminous plants. These contain bacteria (*Bacillus radicicola*) which fix the free nitrogen of the air.
2 The sheath of fungal filaments (*Mycorrhiza*) investing many tree-roots. Tr.

the association of several species. If for example this bacillus is sown in sterilized milk, the latter does not become blue, but only dirty grey. The blue patches only appear in unsterilized milk, for the blue colouring matter can only be formed when an acid is present. The lactic bacteria which are always present in unsterilized milk cause formation of lactic acid, and this enables the blue milk bacillus to produce its peculiar pigment. The addition of acid to the sterilized milk does not answer the same purpose; in that case the blue milk bacillus does not grow, for it cannot stand an excess of acid. In unsterilized milk it uses up the acid as rapidly as it is produced, so that this does not accumulate, and the nutritive medium maintains a favourable reaction. To produce blueness in sterilized milk it is necessary to add a neutral substance, from which the bacillus can gradually draw the necessary acid, for it cannot of itself split up the milk-sugar. If, for example, some glucose, (grape sugar) is added it ferments with production of acid, and the blue colour makes its appearance. The blue milk bacillus is killed by exposure for one minute to a temperature of 176° F. It is, on the contrary, very resistant to 10 per cent solution of

carbonate of soda and 5 per cent solution of caustic soda. It also resists drying very well.

4. **Red Milk.**—Red milk may also be due to the activity of bacteria. I say 'may', because there are cases in which redness of milk is brought about by other causes. It may, for instance, be caused by inflammation of the udder, as a result of which some blood is mixed with the milk. In such a case the milk is red at the time of milking, and a red sediment is formed when it is allowed to stand. If, on the other hand, bacteria are the cause of the red colour this only makes its appearance later on, when these bacteria, which get into it from outside, have had time to multiply and produce the red pigment.

There are several bacteria which possess this power.

(a.) The **blood-red bacillus** (*Bacillus prodigiosus*). This bacillus leads to the production of red patches only on the upper surface of milk, the rest of which is not coloured. It is a short rodlet which does not possess the power of movement. It produces an intense red colouring matter upon other substances, especially slices of potato. A rennet-like ferment is also produced by it, as Gorini has shown. It liquefies gelatine.

(b.) **Rose-red sarcina** (*Sarcina rosea* of Menge). Sown in sterilized milk this microbe multiplies on the surface, and imparts to the cream layer a red colour, which gradually spreads to the rest of the milk.

(c.) **Brown-red sarcina** (*Sarcina rosea* of Schröter). This species of microbe colours milk more of a brownish red.

(d.) **Red-milk bacterium** (*Bacterium lactis erythrogenes*). This was isolated by Hueppe from a kind of red milk. Its length is from $\frac{1}{25000}$ to $\frac{1}{17800}$ of an inch, and its breadth from $\frac{1}{83000}$ to $\frac{1}{62500}$ of an inch. Gelatine is liquefied and coloured red by it. Sown in milk it throws down and peptonizes the casein, the reaction remaining neutral. The cream-layer is not coloured red but only the skim milk below it. The red pigment is only produced when the cultures are made in the dark.

5. **Yellow Milk**.—Several sorts of bacteria can produce a yellow colour in milk. The best known of these is the **yellow-milk bacillus** (*Bacillus synxanthus*, Schröter). This micro-organism was found in boiled milk which had assumed a yellow colour. It is a motile rodlet, which curdles milk by means of a rennet-like

ferment, afterwards redissolving the curd and producing a yellow pigment. It really belongs therefore to the group of casein ferments, and only receives special mention here because of its power of producing colouring matter.

According to my experience there are many bacteria, especially those of putrefaction, which can colour milk yellow. But this rarely appears in practice, for milk is not generally kept long enough for such changes to make their appearance.

6. **Bitter Milk.**—A fairly common fault of milk is bitterness. Often, it is true, this defect is caused by certain feeding stuffs, but there are also certain bacteria which can impart a bitter taste to milk, by producing bitter substances, such as peptone. This property is not, however, limited to a special group of bacteria, for the production of bitter substances, like that of butyric acid, is a feature common to a large number of bacteria and associated with various kinds of fermentation. Bitterness may, therefore, be commonly regarded as a bacterial disease of milk, but the bacteria causing it may belong to various families according to the nature of the bitter substances produced. Most of them belong to the group of casein ferments already described.

Among the bacteria which have been held responsible for this disease are the following:—

(a.) **Weigmann's bitter-milk bacillus.** Rod-lets from $\frac{1}{16600}$ to $\frac{1}{13800}$ of an inch long, and from $\frac{1}{27700}$ to $\frac{1}{22700}$ of an inch broad, feebly motile, and producing spores.

When sown in sterile milk this bacillus gives it a very bitter taste within 24 hours. Cheese does not taste bitter after inoculation with this microbe, which apparently, therefore, is not able to multiply in this dairy-product.

(b.) **Conn's bitter-milk micrococcus.**—This micro-organism has been isolated from a bitter cream. It is aërobic and liquefies gelatine, making it stringy. Milk in which it is sown becomes curdled by the production of a ferment resembling rennet, but is afterwards partly redissolved. The milk does not become stringy like the gelatine, but acquires a very bitter taste.

(c.) **Bitter soft-cheese bacillus** (*Tyrothrix geniculatus*).—This is a bacillus found by *Duclaux* in soft cheese, which, as well as milk, it makes bitter.

(d.) Lastly, a short time since, I succeeded in isolating from a very bitter cheese a micrococcus which gives a bitter taste to milk inoculated with

it. The same bitterness is produced in cheese if a pure culture of this micro-organism is added to the curd. The micrococcus in question liquefies gelatine like *Conn's* micrococcus, but does not make it stringy. Being a member of the lactic ferment group it curdles milk, and at the same time gives it a bitter taste. It grows well in broth and upon agar. Upon potatoes it forms a whitish crust with yellowish edges. On account of its power of making cheese bitter I have named it the **bitter-cheese bacillus** (*Micrococcus casei amari*).

This by no means exhausts, however, the list of microbes which can impart bitterness to milk, for this power is possessed by a great many of them. It is noticeable that a bitter taste is most frequently found in boiled milk which has been allowed to stand for some time. This is because the bitter milk bacteria chiefly belong to the group of casein ferments, as has been already stated. We know that most of these are characterized by great powers of resistance (spores). When, therefore, milk is boiled the lactic bacteria are killed, but the spores of the bitter milk bacteria remain alive, germinate, and multiply luxuriantly. In raw milk, on the contrary, they

are easily choked by the lactic bacteria. There are, however, microbes which can endure the presence of these last and which at the same time possess the power of making raw milk bitter. I have twice succeeded in isolating from bitter cream two sorts of bacillus, not belonging to the group possessing very resistant spores, and yet abundantly developed in milk in spite of the presence of lactic bacteria. They seem not to have been hitherto described, but my study of them is not yet completed.

The subject well deserves special and thorough investigation; for the present we must be content to remember that numerous bacteria can impart a bitter taste to milk, owing to substances which they produce.

7. Stringy or filamentous Milk.—Milk and especially cream often become slimy or stringy within 12 or 14 hours after milking. When this happens a sticky consistency is acquired, which is often so marked that the liquid can be pulled out into a long thread if a finger or a glass rod is dipped into it. This change in milk is another instance of the action of bacteria, and is easily transmitted. As a rule this fault in milk is much deprecated, especially in Switzerland where milk

so affected is not willingly employed in the manufacture of cheese. In some other countries, on the contrary, stringy milk is appreciated and even produced artificially, as, for example, in the manufacture of Edam cheese, and of the 'Tættemælk' (a preparation of stringy milk) of Norway.

A large number of bacteria are able to induce this change in milk. Those which have so far been studied with the most care will be here briefly described.

(a.) *Schmidt* of Mülheim was the first (1883) to study stringy milk bacteriologically. He saw in a sample of such milk an enormous number of micrococci having a diameter of $\frac{1}{25000}$ of an inch. They were motile, and partly isolated, partly arranged in chains. The reaction of the milk was acid. Unfortunately *Schmidt* did not cultivate these microbes, so that it is not possible to identify them with species subsequently discovered.

(b.) *Duclaux* described two species of bacteria, the **slimy-milk ray-microbe**, *Actinobacter*, and an allied form (*Actinobacter polymorphus*), which both make milk slimy. They possess a capsule.

(c.) *Loeffler* has isolated a **slimy-milk bacillus** (*Bacillus lactis pituitosi*), which makes sterilized milk slimy. He describes it as a stout, slightly

curved rodlet, which does not liquefy gelatine. At the temperature of an ordinary room its growth is only moderately rapid, so that it can scarcely play an important part in practice.

(d.) The **viscid-milk bacillus** of *Adametz* (*Bacillus lactis viscosus*). *Adametz* has isolated a bacillus that makes milk extremely stringy, from the water of two brooks near Vienna which carry off the waste from several manufactories. It is a short rodlet of from $\frac{1}{20000}$ to $\frac{1}{14300}$ of an inch long, and $\frac{1}{22700}$ to $\frac{1}{20000}$ of an inch broad. It does not liquefy gelatine and is very aërobic. Sown in sterilized milk it does not produce stringiness till several days have elapsed. At the ordinary room-temperature the milk does not become markedly stringy for a considerable time. This bacillus, therefore, is probably never the cause of stringiness in actual practice, for milk is not kept sufficiently long. For this reason its study is chiefly a matter of theoretical interest.

(e.) *Van Laer* has isolated two kinds of bacillus from stringy butter, both of which possess the power of making milk stringy. He has named these **slime bacillus I and II** (*Bacillus viscosus*, *I and II*). Slime bacillus I forms a very

viscid yellowish-green curd on the surface of milk. Later on the casein entirely dissolves and the milk assumes a greenish tint.

(f.) A considerable number of the **potato bacilli**, which, as already stated, belong to the group of casein ferments, are able to make milk slimy. But they never produce true stringiness of such a kind that the milk can be drawn out into threads.

(g.) '**Long way**' **micrococcus**.—In Holland a stringy kind of skim-milk is used in the manufacture of Edam cheese. From this fluid, known as 'long way', Weigmann has isolated a micrococcus capable of producing this kind of alteration in milk.

It often occurs in the chain-form, on which account it has also been called **Dutch streptococcus** (*Streptococcus Hollandicus*). It does not liquefy gelatine, and when sown in sterilized milk makes it stringy within 12 to 15 hours, at a temperature of 77° F. The milk becomes sour at the same time.

(h.) The clotted milk (Tættemælk) so much consumed in Norway is a preparation of stringy milk. In this case stringiness is produced by adding leaves of the **butterwort** (*Pinguicula*

vulgaris), or by feeding these to the cows. A micro-organism able to make milk stringy has been found on these leaves, and it is perhaps identical with the 'Long way' Micrococcus.

(i.) *Schütz* has also isolated a special micrococcus from stringy milk, but it does not appear to be of practical importance.

(j.) **Guillebeau's c bacillus**, mentioned previously, makes milk extremely stringy. As it is present when the udder is inflamed, the milk is in this case faulty at the time of milking and stringiness is not due to later infection.

(k.) The commonest agent of this defect in milk, at any rate in Switzerland, is undoubtedly a micrococcus discovered by *Professor Guillebeau* of Berne, and named by him **Freudenreich's micrococcus** (*Micrococcus Freudenreichii*). This micro-organism was found near Berne, in a dairy the milk from which had long been stringy. Since then I have again met with it in a very large number of milk-samples from the cantons of Berne and Soleure, so that here at least it may be looked upon as the chief cause of this alteration in milk. It is a large round coccus with a diameter of $\frac{1}{12500}$ of an inch, or more. It is non-motile, and liquefies gelatine. In sterilized milk

it thrives best at a temperature of 68° F., and in a short time induces such extreme stringiness that the milk can be drawn out into slender threads from 1½ to 3 feet long. In unsterilized milk such a change is obvious after five hours.

This micrococcus possesses great vitality. Thus, for example, *Prof. Guillebeau* found it still living in September in milk which had been inoculated with it the previous March. This shows that it finds the conditions of life in cow-houses favourable, and that it is, therefore, difficult to get rid of. Experiments made by *Guillebeau* shew that the microbe when introduced into the udder by inoculation is scarcely able to maintain its existence. It is not, therefore, a pathogenic form, and stringiness is, therefore, but rarely to be referred to disease of the udder, being almost always a fault due to infection during or after milking. The microbe is killed by exposure for two minutes to the temperature of boiling water, but can, on the other hand, support drying for three days. In Switzerland stringy milk is excluded from the cheese manufacture. I have not, it is true, been able to prove that this microbe produces cavernous structure—which, however, is not very likely, since it does not cause the liberation of an

appreciable amount of gas,—but it is easy to imagine that stringiness would prevent the cheese from being of uniform texture, and lead to the production of ‘nests’. Should this disease make its appearance in a cow-keeping establishment suitable disinfective measures must be employed. These will be dealt with in detail later on, under the head of disinfection.

(1.) **Hess's bacterium** (*Bacterium Hessii*).—The same investigator has found in the milk of a cow from a mountain pasture in Emmenthal (canton of Berne) a second microbe, which also possesses the power of making cream slightly stringy. This is an actively moving rodlet, from $\frac{1}{8300}$ to $\frac{1}{5000}$ of an inch long and $\frac{1}{20000}$ of an inch broad, which is also able to make broth very stringy, and liquefies gelatine. But as soon as this bacterium produces a little acid at the expense of the milk-sugar, the sliminess of the milk disappears. In dairies, therefore, it cannot produce permanent stringiness.

It thus appears that numerous observations have been made on stringy milk and on the bacteria which cause it, though the exact nature of the process is still but little known. Further investigations must furnish an accurate explanation

of the changes which take place. In some cases the bacteria may so alter the components of the milk that it becomes stringy—in other cases they may perhaps secrete a slimy substance (the formation of capsules in *Duclaux's* Actinobacter supports this) which mixing with the milk gives it a slimy or stringy consistency.

8. **Soapy Milk.**—*Weigmann* has studied a special alteration of milk which makes it 'soapy'. Milk which at first appears normal acquires a disagreeable taste of soap within 24 hours. *Weigmann* has discovered that the cause of this change is a special bacillus, which sown in milk gives it the same soapy taste. He also found this microbe in the straw used as litter, from which it appears that the milk is infected when the litter is changed at milking time. It is, therefore, recommended not to feed straw to cows, nor change their litter, at the time of milking.

OTHER MICRO-ORGANISMS FOUND IN MILK.

So far we have only been concerned with the commoner bacteria found in milk. But there are certain yeasts and moulds which are able to modify it in various ways.

Most of the **yeasts** which get into milk do

not bring about any noticeable alterations. Of late years, however, several have been discovered which possess the power of fermenting milk-sugar, with formation of alcohol and carbonic acid gas (carbon dioxide). Such a form was first described by *Duclaux*, who gave it the name of **milk yeast** (*Saccharomyces lactis*). Later on *Grotenfelt* discovered another yeast which curdled milk and led to the production of alcohol, though only in very small quantities. *Duclaux's* yeast, on the contrary, produces a large amount of alcohol and but little lactic acid. *Kayser* has also described a yeast which strongly ferments milk. *Adametz*, *Weigmann*, and *Mix* have studied similar yeasts. Another example is the yeast found in kephir. This is a native beverage of the Caucasus, where it is made with fermented milk and resembles koumiss. Kephir is made with cow's milk, koumiss with mare's milk. The agent of fermentation is a special fungus, the kephir 'grains', which are allowed to swell up in milk that when afterwards kept in well-closed vessels undergoes a characteristic fermentation. The latter is due to the activity of micro-organisms contained in the kephir grains, and among these is the yeast already alluded to. It is this which transforms

the milk-sugar into alcohol and carbonic acid gas. But it is not able to do this unaided, and is dependent on the help of certain bacteria which modify the milk-sugar in such a way that the yeast can attack it, and at the same time set up lactic fermentation. Kephir affords, therefore, a new example of the symbiosis of organisms alluded to previously, the end-products here being chiefly alcohol, milk-sugar, and carbonic acid gas. Kephir is a refreshing beverage, and useful for many complaints.

Another kind of yeast was discovered by *Dr. Schaffer* on cheeses which were covered with red blotches. This has been studied by *Demme* and named by him **red yeast** (*Saccharomyces ruber*). The five cow-stalls which were found to be the cause of this disease were thoroughly disinfected with sulphuric acid, and after this the trouble was at an end.

Certain **moulds** are also found in milk, and some species are even used in cheese-making. For example, **green mould** (*Penicillium glaucum*), a very widely distributed form, the greenish crusts of which so often invade bread, preserves, &c., is one of the chief agents in the ripening of Roquefort cheese. It is cultivated in large

masses on slices of bread, scraped off, and mixed up with the curd. This mould gives to the cheese its special flavour, and the well-known greenish veins are made up of it.¹

Moulds similarly play an important part in regard to some soft cheeses, such as Brie-cheese. Their growth is encouraged upon the surface of such cheeses, into which the special ferments produced by them penetrate, and which are therefore not washed and wiped like the hard cheeses of Emmenthal or Gruyère. In these latter cases the action of moulds would alter the special flavour, and the surface of the cheeses is therefore kept as clean as possible.

Yet another mould deserves mention on account of its frequent presence in milk and dairy-products, viz. **Oidium lactis**, already briefly described in the general part of this book. When milk is left to sour a thick skin is often formed on the surface in a few days, which is almost entirely composed of the hyphae and spores of this mould.

¹ The flavour and colour of Gorgonzola are also largely due to species of *Penicillium*. Tr.

FEEDING STUFFS AND DEFECTS IN MILK.

Although in what has gone before we are far from having passed in review all the microbes which influence milk in one way or another, yet sufficient examples have been given to show what damage to dairying these minute organisms are in many cases able to effect, and it now remains to consider the means by which we can defend ourselves against them. And with this end in view it is first necessary to examine how far there may be a connection between feeding-stuffs and the diseases of milk and cheese. Here in Switzerland the opinion is very widely spread that these diseases depend upon the fodder, and this is blamed when cavernous structure, stringiness, &c., make their appearance. So far as alterations of milk are concerned which exist at the time of milking, *e.g.*, certain cases of bitterness, it is certainly possible that the feeding may be the *direct* cause of the alteration. But when alterations are in question which only appear some time after milking, or it may be after the making of a dairy-product, as, for example, cavernous structure in cheese, blue milk, &c., then, as we have seen, the results of bacteriological

research prove conclusively that bacteria alone are the *direct* cause, and that it is a mistake to credit the food with producing such effects.

It may be admitted, however, that fodder exerts an *indirect* influence in these processes, for we must not forget that it may be the means of introducing harmful kinds of bacteria. Thus, when cows are fed with the refuse from distilleries (distillery draff), the microbes in which this abounds pass into the air of the cow-house. They can also pass uninjured through the digestive organs of the cow and get into the milk with fragments of dung. Another example has already been given in treating of stringy milk, which in certain countries is obtained by adding to the fodder leaves of butterwort, upon which are found bacteria which can bring about this change. These microbes then get into the milk with dust or dung and make it stringy. In this way may perhaps be explained the appearance of a defect in the milk after change of fodder, but bacteria are always the direct cause.

PRESERVATION AND STERILIZATION OF MILK.

We have seen that milk always contains germs which will spoil it sooner or later, the time depending

upon the temperature and upon the number present. It has also been seen that disease microbes are often present in milk, sometimes from the first, as in the case of tubercle bacilli, and sometimes, like the bacilli of typhus, cholera, &c., introduced later on. These last may, it is true, be for the most part avoided by proper construction of cow-houses (measures being taken to prevent liquid manure from soaking through into brooks, &c.) and by observing scrupulous cleanliness. All these means, however, though they greatly reduce the number of ordinary bacteria of milk, thus improving its keeping power and reducing the chance of defects, do not prevent us from often feeling the need, especially in trade, of milk which will keep still better and which has been absolutely freed from disease germs which it may possibly have contained. Health specialists, not without reason, are constantly making an outcry on this score. But these two desirable conditions—absence of disease germs and prolonged keeping powers—are not identical. Thus, for example, milk may be made incapable of communicating disease, without giving it unlimited keeping power, for it is generally easier to destroy disease germs than the ordinary bacteria to which the common changes

of milk are due. On the other hand sufficient keeping power may be conferred without destruction of disease germs at the same time, as, for example, when cold is employed as a preservative. It is therefore necessary to examine in every case, how far a given method satisfies the two conditions. As to preservation of milk a distinction is drawn between **sterilization**, by which unlimited keeping powers are conferred, and such methods as **pasteurization**, which only keep the milk good till it reaches the consumers.

To attain the double end there is only one means, *i.e.*, destruction of the ordinary bacteria at the same time as the pathogenic ones, or at least to check their action, since they are responsible for the changes which take place in milk. How then can this be effected? There are only two kind of agents to be considered,—physical agents and chemical agents. We will begin with the latter.

I. CHEMICAL AGENTS OF PRESERVATION AND STERILIZATION.

We have already seen that all substances which are poisonous to living protoplasm are so to bacteria as well. Such substances when added

to milk will therefore kill the contained microbes, but it is obvious that those which are injurious to health must not be used, as milk to which they have been added can no longer be consumed without danger. It is therefore necessary to exclude all poisons, such as corrosive sublimate, carbolic acid, &c.

The chief substances which it appears possible to use may be placed under three headings:

(a.) Substances which may be expected, owing to their alkaline nature, to neutralize the acid which is produced in milk and thus to hinder curdling (alkaline salts, carbonate and bicarbonate of soda).

(b.) Substances which without being dangerous can either kill organized ferments, or else check their action (*e.g.*, salicylic and boracic acids).

(c.) Substances which can do both (*e.g.*, borax, quicklime).

Lazarus has published his very careful researches on the use of chemicals, and what here follows is extracted from these.

It is clear that these substances cannot be added to milk except in quantities which will neither affect its taste nor make it harmful, especially for children and sick persons. As the *maximum*

amounts allowable *Lazarus* gives the following :

	<i>per quart.</i>
Carbonate of soda	48 grains.
Bicarbonate of soda.	48 „
Boracic acid	16 to 32 „
Salicylic acid	12 „
Borax	64 „
Quicklime.	24 „

It is to be remarked, however, that these amounts conflict with most police regulations, and further that certain much lauded methods of preservation receive attention here more on account of their theoretical interest than because results of practical importance follow from them. We shall, on the contrary, see that the results obtained by *Lazarus* tell for the most part against the use of chemical preservatives.

In his experiments *Lazarus* proceeded in the following way. The milk, drawn from the cow in his presence, was mixed with the substances of which he wished to test the action, divided into portions of about $\frac{1}{2}$ a fluid-ounce each, and placed in test-tubes. It was then sterilized and inoculated with pure cultures of various bacteria (part of a broth-culture added to each tube in the proportion of 1 per cent of its volume). The same

thing was done with milk to which these substances had not been added, and with raw milk milked as cleanly as possible into sterilized vessels but not itself sterilized. Immediately after inoculation the number of bacteria introduced was estimated, after which the test-tubes were kept at various temperatures (72° F. and 95° F.) and the number of bacteria estimated again three, six, nine, twelve, and twenty-four hours later. In this way it was possible to determine whether the addition of a given chemical did or did not check the growth of microbes.

1. **Experiments with carbonate and bicarbonate of soda.**—*Lazarus* found, in the first place, that these substances had been added to 40 out of 64 samples of milk bought in the market, showing that especially in large towns dairymen frequently have recourse to this procedure, hoping thereby to increase the keeping power of their milk. Let us now see the result of the investigation. In sterile milk souring was delayed from 6 to 12 hours at 95° F., and from 12 to 20 hours at 72° F. In unsterilized milk, on the contrary, curdling took place at about the same time as a rule in chemically treated samples and those not so treated. This is due to the

presence of rennet-producing bacteria, the growth of which is favoured by alkalies. It, therefore, follows that what is gained in one direction, by checking the action of the lactic bacteria, is lost in another direction, by promoting the growth of rennet bacteria, so that the keeping power of the milk is not increased. As to pathogenic bacteria, these are not injured by these particular preservatives; on the contrary, the growth of cholera bacteria, which prefer an alkaline liquid, is even promoted by them. Alkalies, therefore, when the permissible quantities are used, are powerless against disease germs and of very doubtful value as preservatives.

2. **Salicylic acid.**—A taste is given to milk by this compound when the amount added is only 12 grains per quart. It hinders curdling more than other substances. Thus, the curdling of sterilized milk inoculated with *Hueppe's* lactic bacillus, is retarded for two or three days. Cholera bacilli are killed in 6 to 24 hours, according to the temperature—but not typhus bacilli, the growth of which is scarcely hindered. Salicylic acid added in such amounts does not, therefore, give complete security against disease germs, besides which specialists are not agreed as to its

harmlessness. In France especially doctors oppose its employment in the preservation of food, believing that its constant use is injurious to health.

3. **Boracic acid.**—According to *A. Mayer* this acid makes milk keep very well. The experiments of *Lazarus* show, on the contrary, that it has little power against germs, and that milk to which it is added curdles as soon as ordinary milk. Against disease germs especially it proved quite powerless.

4. **Borax.**—This has only a feeble action against bacteria, but is, however, able to retard curdling for 24 hours. The number of disease germs is scarcely reduced.

5. **Quicklime.**—Generally speaking this substance possesses very powerful germicidal properties, which make it extremely useful as a disinfectant, so it might be expected to have a preservative action on milk. *Lazarus* has found it, on the contrary, quite useless for this purpose, which is explained by the fact that the carbonates and phosphates of the milk unite with quicklime to form inactive compounds.

6. **Hydrogen peroxide.**—According to *Heidenhain* the addition of one part of hydrogen peroxide to 10 parts of milk extends its keeping

powers for from 3 to 8 days. Such amounts cannot, however, be employed in practice, besides which the hydrogen peroxide of commerce is seldom pure, generally containing poisonous barium chloride.

To sum up—the addition of these various chemicals, even if it improves the keeping power of milk, is not to be recommended, for it is very difficult to be perfectly sure that a substance which at first sight seems innocuous is not injurious to health when used for a long time. If therefore seems better to prevent the misuse of such preservatives by simply prohibiting their employment.

PHYSICAL AGENTS OF PRESERVATION AND STERILIZATION.

1. **Filtration.**—Purely mechanical methods of purification, such as filtration through porous earthenware (Chamberland and Berkefeld filters), by which germs can be removed from liquids, cannot be employed for milk, because the filter keeps back the fat-globules and the suspended casein. The filtered liquid is therefore nothing but whey.

2. **Electricity.**—It is possible that one of the

numerous applications of electricity may give us in the future a means of destroying micro-organisms contained in a liquid without otherwise altering the latter. Up to the present time, however, no facts are known which warrant the hope of a speedy solution of this problem.

3. **Cold.**—We know that a low temperature is unfavourable to a rapid increase of bacteria, and this is why milk is to some extent preserved by cooling it rapidly after milking. Notwithstanding this, the hope has not been realized that by refrigeration milk might be made to bear transport for long distances, and even sterilized. The first objection to the method is that it raises the cream, so that cream and skim milk freeze separately, and do not properly mix again on thawing. Besides this, any disease germs which may be present in the milk are not killed by this procedure, for as we have seen certain pathogenic bacteria can withstand a temperature of -202° F. Such milk, therefore, does not meet the requirements of hygienists.

4. **Preservation by Heat.**—This has always been a common way of making milk keep better. Every housewife knows by experience that milk which has been boiled and cooled again keeps

much longer than raw milk. This fact is easy to understand after what has been said about milk bacteria. In fact boiling destroys most of the germs, and the milk does not spoil till those which withstand it have had time to multiply anew to a sufficient extent. There are, however, two objections to this method: in the first place the taste of the milk is somewhat altered, especially if it is boiled over an open fire—and part of it evaporates during boiling so that the composition is modified. To this must be added that boiling does not give milk unlimited keeping power, since numerous bacteria can endure the treatment. The milk can be prevented from burning by heating it in a water-bath, and pieces of apparatus have been devised constructed on this principle, with the special view of preparing approximately germ-free milk for the use of children. The best known are those of *Dr. Egli-Sinclair* and *Soxhlet*. They consist of a tin receptacle filled with water, which is put on the fire and which holds a number of small bottles that are heated in the boiling water for about a quarter of an hour. Each bottle holds enough for a child's meal. After heating, these bottles are cooled and ingenious methods of closing them have been invented. They are not opened

till the milk is wanted, so that in the meantime no bacteria can get in from the outside, as is the case when all the milk is boiled in a single vessel, which has then to be opened several times a day. These little pieces of apparatus are generally called **milk-sterilizers**, though the milk is not made absolutely sterile or germ-free. The chief advantage of the method is that at least all the pathogenic bacteria are killed by it. Attempts have been made in two different directions, to perfect the methods of heating milk, the aim of one being to secure absolute freedom from germs, of the other to preserve the natural taste, a matter of great importance when the milk is destined to be made into butter. The two methods are known as *sterilization* proper, and *pasteurization*.

(a.) **Sterilization**.—By this is meant, as already stated, a process which ensures the absence of all germs in, *e.g.*, a liquid. To make milk *absolutely* germ-free is no easy matter, owing to the very resistant nature of some of the bacteria which it always contains, and to attain complete success it is necessary to expose it for about a quarter of an hour to a temperature of 230° F. to 239° F. This can be done either in large steamers, hermetically sealed and heated on the fire, or in special

pieces of apparatus into which steam of the required temperature can be conducted. Such apparatus has, of course, to be constructed in such a way as to withstand pressure. The inconvenience of this method lies in the fact that exposure to these high temperatures alters the milk even more than mere boiling. It becomes brownish and acquires a burnt taste, owing to the action of the heat on the milk-sugar. Adults, being used to the taste of fresh milk, seldom get accustomed to the burnt flavour, and up to the present milk thus sterilized has been chiefly used for feeding children, for whom absolute freedom from germs is the chief requirement. The process has now, however, been modified and improved in various ways, with a view of obviating too much alteration of the milk.

In the first place stress is beginning to be laid on **cleanly milking**. In fact, it is much easier to sterilize clean milk, even at less elevated temperatures, than milk which swarms with bacteria owing to inadequate attention to cleanliness at the time of milking. Not only the nature but the *number* of bacteria play an important part in this connection. It has also been noticed that the milk often gets infected at the moment of closing the bottles, to obviate which arrangements have

been devised by which they are closed while still under pressure, and before removing the lid of the sterilizer. Such an apparatus is used for example at Stalden (canton of Berne), by the recently established Milk-sterilizing Association (*Neuhauss-Groenwald-Oehlmann* process).

Thanks to these and other technical improvements it appears possible to obtain, almost always, perfectly sterilized milk without employing so high a temperature as 239° F. At any rate the spores which resist the temperatures employed are so enfeebled that they rarely germinate, especially if the bottles are kept in a cool place. Milk so prepared has already stood the test of long journeys without spoiling. As already stated cleanly milking here plays an important part. It is scarcely necessary to emphasize the importance of finding a means of preserving milk in a faultless manner. In many countries there are no milch kine, so that it is impossible to procure this important food, while in large towns the difficulties in the way of an adequate milk-supply often favour adulteration. These evils would be mitigated were a milk prepared which could stand long journeys. At the same time this new industry would prove a constant source of revenue to

milk-producing countries, and open new channels of trade for their milk. It is to be hoped, therefore, that we may succeed in removing the defects of the present method, which, as previously stated, lie in this—that when absolute sterilization is the chief aim the taste of the milk suffers, while the employment of more moderate measures lead to uncertainty as regards sterilization.

(b.) **Pasteurization of milk.**—In cases where it is sufficient to make sure of milk keeping for a limited time, and where it is of special importance to alter neither its taste nor appearance, pasteurization may be employed with advantage.

We have seen in the first part of this book that a temperature of 140° F. to 158° F. is sufficient to kill most adult bacteria, the spores only remaining alive. *Pasteur* introduced a process, to which his name has been given, for preventing certain defects of wine and beer and which consists in heating these liquids to about 140° F. But when wine and beer are in question the task is easier than in the case of milk, because these liquids are not such good feeding grounds for most bacteria as the latter fluid, and when they have been pasteurized the germs which have withstood the process multiply less readily than

those remaining in pasteurized milk. Nevertheless it was proved by the first experiments that the process considerably increases the keeping power of milk. It would carry us too far to describe all the apparatus that had been devised for pasteurizing milk, and reference may be made in this connection to an excellent little work published in German by *Dr. Weigmann*, which treats very thoroughly of this subject.¹ Pasteurizing apparatus is in general of two sorts: that in which the milk is exposed for a short time only to a temperature of 158° F. to 167° F. and that in which it is heated for a longer time to a temperature somewhat under 158° F. As an example of the first kind of apparatus that of *Thiel* may be mentioned, which is resembled by quite a series of others. In this particular arrangement the milk runs slowly in a thin layer down a heated wavy surface and then enters a cooler after it has been heated to a temperature of from 158° F. to 167° F.

Fleischmann has made a number of experiments on the keeping power of milk thus treated, and finds that if afterwards maintained at a temperature

¹ Dr. A. Weigmann,—*Die Methoden der Milchconservirung, speciell das Pasteurisiren und Sterilisiren der Milch*. Heinsius, Bremen, 1893.

of 54° F. to 57° F., it keeps at least 30 hours longer than ordinary milk. *Van Geuns* states that he observed such milk, when kept at 50° F. to 54° F., turn sour from 1 to 3 days later than ordinary milk.

As to the persistence of disease germs in such milk, the experiments of *Lazarus* have shown that cholera bacilli but not typhus bacilli are killed in *Thiel's* apparatus at a temperature of 143° F. to 158° F. It was only by raising the temperature to 167° F. that the latter were generally destroyed. The fault of this kind of apparatus lies in the fact that the milk in running over the metallic surface is heated for too short a time. Of late years, therefore, *Thiel's* apparatus and others of the same kind have been replaced by arrangements of a different sort, in which the milk is kept for a longer time at the required temperature, which is not allowed to exceed 154° F. to 156° F., for, as *Duclaux* has shown, the temperature of 158° F. is precisely the one at which the change in taste, which it is desirable to avoid, begins to appear. The milk in apparatus of the kind is generally heated by steam, and is kept in movement by special means, so that it does not acquire a burnt taste. *Bitter* has very carefully investigated this

new method of pasteurizing milk, and proved its efficiency. He first endeavoured to determine whether by it disease germs are destroyed with certainty. The results attained were as follows.

Bacilli of Tuberculosis.—Milk was mixed with bacillus-containing expectorations from consumptive patients, and heated for various periods to 154° — 156° F. Guinea-pigs, which are very sensitive animals as regards tuberculosis were then inoculated with this milk. It was thus shown that after 20 minutes treatment the milk was no longer virulent, while animals inoculated with a *non*-pasteurized portion of the same milk all contracted tuberculosis.

Typhus.—In this case *Bitter* added pure cultures of typhus bacilli to the milk, so that every 10 drops of it contained 1 million bacilli, a number far above that likely to be met with in practice. Furthermore *Bitter* always used 9 gallons of milk for each experiment, so as to anticipate the objection that investigations made on a small scale do not give results applicable to the larger operations of trade. After pasteurization he determined by the method of culture whether the bacilli still remained alive, adding portions of the milk to nutritive media of suitable kind. In this

case animals could not be employed, as none are known which can be successfully inoculated with typhus. He proved that heating for 15 minutes to 154° — 156° F. kills the typhus bacillus with certainty.

Cholera and Diphtheria Bacilli are less resistant than those of tuberculosis and typhus. It may, therefore, be supposed that they too would be killed by this treatment.

Other Disease Germs.—It was, of course, not possible to experiment on scarlet fever, measles, and other similar diseases of which the agents are not yet known. If, however, a sample of milk should contain their supposed microbes, it might be reasonably expected that they would be killed by pasteurization, for experience shows that in these diseases the disinfection of dwellings, &c., is not more difficult than in other cases.

It therefore follows from these researches that pasteurization for 20 to 30 minutes, at a temperature of 154° — 156° F., kills with certainty all the disease germs that are liable to be found in milk. Such a milk therefore fulfils all the requirements of hygiene.

Let us now see how saprophytes, to which group the ordinary milk bacteria belong, support

this temperature, in other words what is the keeping power of pasteurized milk? In order to determine the number of bacteria resisting pasteurization *Bitter* estimated their number before and after the process. He found, in the first place, that heating for 20 minutes diminished their number as much as heating for 35 minutes. In practice, therefore, 20 minutes may be regarded as sufficient. The following figures prove the efficacy of the process.

They give the average number of bacteria found, for each case, in 10 drops of milk.

Sample.

1.	Before	pasteurization,	102,600;	after,	2	to	3
2.	"	"	251,600;	"	30	"	40
3.	"	"	25,000;	"	3	"	5
4.	"	"	37,500;	"	2	"	5
5.	"	"	94,000;	"	2.		

This kind of pasteurization gives better results, therefore, than that effected in *Thiel's* apparatus. *Van Geuns*, who worked with the latter found that milk pasteurized in it still contained 5,000—9,000 bacteria, in every 10 drops.

A series of 19 experiments conducted by myself confirm for the most part the results of *Bitter*. As, however, my work was done in

summer with ordinary market milk, while *Bitter* pasteurized the milk immediately after milking, and as I, therefore, experimented with a fluid richer in bacteria my figures are on an average somewhat higher. As a rule I found after pasteurization from 10 to 40 bacteria per 10 drops, and sometimes from a few hundreds to a thousand. The differences in the results are easily explained, for the number and nature of the milk bacteria constantly vary. On one day the germs present may be very numerous and very resistant, on another day the contrary may be the case. In order to find out how far the keeping power is increased by the treatment, *Bitter* exposed samples of pasteurized milk to various temperatures, considering it as turned when it would no longer stand boiling without curdling. Here again it appears that temperature plays an important part. At temperatures above 86° F., and, therefore, most favourable to the increase of the bacteria remaining alive, the keeping power was not extended for more than 6 to 8 hours. At 77° F., it was extended for at least 10 hours, at 73° F., for 20 hours, and at 57°—59° F., for 50—70 hours. This sufficiently proves the necessity for rapid cooling after pas-

teurization. It also makes a great difference whether the pasteurized milk is kept in sterilized or non-sterilized vessels. The following figures furnish instructive examples.

When kept at 68°—75° F. the milk turned—							
<i>in sterilized vessels</i>				<i>in non-sterilized vessels</i>			
Expt.	1	after	46 hours		after	24 hours	
"	2	"	96	"	"	48	"
"	3	"	72	"	"	24	"
"	4	"	130	"	"	65	"
"	5	"	86	"	"	48	"
"	6	"	104	"	"	66	"
"	7	"	46	"	"	18	"
"	8	"	80	"	"	48	"

These eight experiments show that it is impossible to be too cleanly, or even too fastidious, in the treatment of milk, since the best rules become useless if the smallest negligence is permitted. It should not, therefore, be forgotten how important it is to scald with steam or boiling water all utensils and other objects that will come into contact with the milk. The experiments of *Bitter* promise results useful in practice. At 68° F. raw milk, which has not been pasteurized, is spoilt within 20 hours, and in 15 hours when kept at 80°—86° F. Pasteurized milk on the contrary

should be as good as ever after 30 hours, supposing for example that it remains for 10 hours before transport at the temperature of the cellar, that other 10 hours are then spent in transit at a temperature of 71° — 73° F. (for this purpose the cool night can be utilized), and that 4 or 5 hours more elapse before it passes from the market into the hands of the consumer, who keeps it a few hours longer before using it. *Bitter* proved by the following experiment that this view would be realized in practice. He kept a sample of pasteurized milk for 10 hours at 57° F., for 22 hrs. longer at 73° F. and lastly for 7 hrs. at 86° F. It was then absolutely sound, and kept good for 7 hrs. longer at 73° F.

It is possible that later on cream also will be submitted to pasteurization, at least in dairies where defects frequently occur. This might be done in view of transport for long distances, or in connection with butter-making, to which pasteurized cream lends itself very well. The yield of butter is perhaps rather less but this disadvantage would be made up for by the improved quality of the product.

Another important application of pasteurization has been spoken of earlier, in dealing with *Weig-*

mann's process for ripening by means of pure cultures cream intended for butter-making.

Lastly it may still be added that pasteurized milk curdles very readily with rennet, and can therefore be used for making cheese. In this way it is possible to study the action of various bacteria on the ripening of cheese, since pasteurization destroys most of the milk bacteria and it is then only necessary to add pure cultures of those bacteria the action of which is to be studied.

RULES TO BE FOLLOWED IN CASES OF MILK DEFECTS.

In the previous sections we have learnt to recognize the source of many defects in milk, and thanks to that knowledge are therefore in a position by the use of suitable means (cleanliness, &c.) to prevent in some measure the appearance of these defects, a procedure which is always easier than to get rid of them when once established. But diseases of milk sometimes make their appearance, even when all the rules suggested by prudence are followed, just as we are ourselves constantly exposed to the attacks of various diseases in spite of proper nourishment and observance of the laws

of health. What is to be done then if such a disease makes its appearance in a cheese-factory or dairy? In the first place it is necessary to discover the cause of the mischief, and then to remove the conditions which favour it. When large quantities of mixed milk are used, a careful investigation of all the cow-houses must be made, with the view of discovering the source of the contaminated milk. Since, too, it happens, as we have seen, that in cases of inflammation of the udder the milk contains undesirable micro-organisms which cause cheeses to heave, it is necessary at the same time to have the cows inspected by a veterinary surgeon.

According to the nature of the disease the examination of the milk will be less or more difficult; in the latter case where it is necessary to isolate and cultivate bacteria, or else to have recourse to accurate chemical analysis, the examination can only be conducted in a properly equipped bacteriological or chemical laboratory, to which samples of the suspected milk should be sent. These samples must be put in bottles which have been sterilized, or at least washed out several times with boiling water, and if the distance is great it is well to send them packed

in ice so as to prevent the bacteria from multiplying too rapidly. In other cases the examination can easily be made at the cheese-factory or dairy.

If, for example, heaving of the cheeses makes its appearance it is necessary to find out which milk contains the bacteria of the disease, bacteria which are now partly known to us. A well-known and very simple test is that devised by *Walter*, cantonal chemist at Soleure, and termed the 'fermentation test'. The milk, placed in glasses, is exposed to a uniform temperature of about 98° F., and if good should remain for 10—12 hours without curdling or undergoing abnormal fermentation.¹

This fermentation test, it is clear, is entirely a bacteriological one. In making it we start with the assumption that if the milk contains too large a number of bacteria of gas-producing kinds, these will be able to make their objectionable action apparent with the help of the favourable temperature employed. In fact the milk thus

¹ Very good 'milk fermenters' have been constructed with which to make this test. They consist of a water bath and spirit-lamp, together with the necessary glasses and lids, and can be procured from H. Dinkelmann, Eisenhandlung, Berthoud, Berne canton, Switzerland.

tested is often seen to curdle and present obvious signs of heaving, from which it is concluded that it is unfit for cheese-making. To get reliable results from this method of procedure it is necessary to make sure that the glasses for holding the milk have previously been sterilized. We have learnt, in connection with the pasteurization of milk to realize the influence of sterilization in the milk vessels, and have seen that pasteurized milk keeps almost twice as long in sterilized vessels as in unsterilized cans. If, therefore, in using the fermentation test we place a milk which is perhaps quite sound, in non-sterilized glasses, these may have many noxious bacteria clinging to them, which set up fermentations that would not otherwise make their appearance. The best plan is to sterilize the glasses for half an hour by means of steam. This is easy to do in cheese-factories and in dairies where steam is used. A metal box (copper or sheet-iron) in which the glasses can be placed will answer the purpose. This is connected by a tube with the steam-engine in such a way that steam passes in below and out again by an opening above. Where such a sterilizer cannot be contrived it may suffice to sterilize the previously well-cleansed glasses by

washing them out with concentrated sulphuric acid, afterwards rinsing them several times in succession in water which has just previously boiled for a quarter of an hour. The lids must also be sterilized, which is most easily managed by holding them for a few seconds over the flame of a spirit-lamp just before covering the glasses with them. In a sterilizer of the kind described, quite a stock of glasses can be prepared in advance, the apparatus of course remaining closed till they are wanted, and a cover being placed over the hole serving for the escape of steam, lest dust should fall in. If the glasses have to be sterilized with sulphuric acid this must be done immediately before they are used. The glasses should all be filled to the same extent, then placed in the water-bath regulated for 98° F., and examined from 10 to 12 hours later. If several samples have to be examined at the same time care must be taken not to ladle them into the glasses with the same spoon or other implement, as this would transfer bacteria from one sample to another. In sending samples of milk to a laboratory the bottles must previously be sterilized in the way described above.

It may happen that milk examined in the way

described turns out perfectly sound, but that in spite of this the cheeses heave which are made from it. In such cases the fault may perhaps lie in the rennet employed, or in the water used for washing the utensils in the cheese factory,¹ for both rennet and water as well as milk are liable to be contaminated by harmful bacteria. Thus, for example, *Adametz* cites a case where in a cheese-factory only those cheeses heaved which were prepared with a certain rennet, while others made from the same milk and in the same way, except that another sort of rennet was used, were all right. Under such circumstances it is, therefore, necessary to examine the rennet and the water for bacteria, which too can be conveniently done by *Walter's* fermentation test. Some sterilized or at any rate well boiled skim milk is taken, and placed in the milk-fermenter, a little of the

¹ In many Swiss cheese-factories the practice is followed of adding a certain quantity of water to the milk before making it into cheese, the proportion being about 40 to 80 quarts of water to 1000 quarts of milk. The reason for this is not very clear; the cheesemakers usually allege that in certain districts the cheeses ripen badly if it is not done. Is it perhaps the introduction of a certain number of bacteria with the water that helps ripening? In any case it is clear that the good quality of this water is not a matter of indifference.

suspected rennet or water being added. As in all cases the apparatus is kept at 98° F.

In order to determine more accurately the amount of contamination, some glasses are inoculated with one drop only, others with from several up to about 60 drops. If obvious fermentation takes place in the glasses inoculated with only one or a few drops, the rennet or water may be considered suspicious. If the rennet is faulty it must be replaced by another, if the water is suspected the source of contamination must be sought for.

When a sample of milk curdles badly on the addition of rennet, the cause is to be looked for in the chemical composition of the milk rather than in the action of bacteria. In such cases the milk supplied from different sources can be tested at the cheese-factory itself by means of the 'milk-curdler' of *Dr. Schaffer*, cantonal chemist at Berne. For this purpose the milk is heated in a water-bath¹ to 95° F. and treated with a given quantity of rennet. It is then seen whether it curdles within the usual time. To $\frac{1}{5}$ of a pint (4 fluid ounces) of milk is added $\frac{1}{50}$ its volume ($\frac{2}{3}$ of a

¹ This apparatus can be bought from G. Joho, 20 Zeughausgasse, Berne, Switzerland.

drachm) of a solution of rennet prepared by dissolving one of *Hansen's* smallest rennet tablets in $\frac{9}{16}$ of a pint (18 fluid oz) of water. Sound milk ought to curdle in from 10 to 20 minutes. If milk thus tested curdles badly or not at all, it should not be employed for cheese-making.

If stringy milk makes its appearance, the source of supply must be ascertained by putting samples on one side and examining them in from 12 to 24 hours afterwards. The microbes which cause this defect are mostly found in the cow-houses and get into the milk at the time of milking. This is easily proved by leaving uncovered glasses of milk in the cow-house. If, on the contrary, the udder is carefully cleansed and a little milk drawn into glasses which are then quickly covered, the milk does not become stringy or only slightly so, because it is not sufficiently contaminated. But in cases of inflamed udder caused, for instance, by *Guillebeau's* c bacillus, the milk is already stringy when first drawn. The reason for this being, therefore, known, the above test is unnecessary.

When the cow-house is proved to be the source of the bacterial disease of the milk it must be **disinfected**,—*i.e.*, freed from the noxious bacteria which it harbours,—so as to put an end to the

disease. There are a host of disinfectants but we may exclude those which are either difficult to apply or dangerous on account of their poisonous nature. Those to be chiefly recommended are milk of lime and also sulphur, a substance in common use for such purposes. Every disinfection of a cow-house should be preceded by a thorough cleaning. After the animals have been turned out all dirt must be removed, and the walls, roof and floor first scraped and then vigorously purified with water and brush. Then the true disinfection can be begun. If burning **sulphur** is used, all openings by which the fumes could escape must, of course, be closed and paper stuck over the joints of the windows and other crevices. Nor must the sulphur be used in a niggardly way. According to the experiment of *Thoinot* and others at least from $1\frac{1}{4}$ to $1\frac{1}{2}$ ozs. per cubic yard should be burnt. I have generally found, however, that $\frac{1}{4}$ of an oz. per cubic yard is sufficient in practice, if by careful scraping and washing most of the agents of infection are previously removed. The best method is to pound up some roll sulphur and then burn it in an iron pan with a little wood and paper. As soon as it is alight the door must be closed and made air-tight by

sticking paper over the cracks, after which at least from 12 to 24 hours must be allowed to elapse. Meanwhile the cows should be thoroughly cleaned, for numerous microbes always cling to their coats, and special care should be taken to clean their feet with soda and water applied with a brush. The clothes of the milkers must also be purified and all the utensils which have come into contact with the milk must be scalded out. After disinfection the cow-house must be well aired.

According to the experiments of *Giaksa* and *Cronberg* walls and floors infected with bacteria can easily be sterilized by washing them over with concentrated **milk of lime**. Only certain very resistant bacteria can survive this treatment. The process is very simple. Some quicklime is dissolved in a little water and the solution mixed with the same bulk of water (50 per cent milk of lime). Walls, roof and floor are then washed over with it, of course after previous cleaning. Although I have not so far had occasion to test this method practically, yet its simplicity and safety appear to me very strong recommendations.

In cheese-factories and dairies where steam is

available this may advantageously be employed instead of boiling water for disinfecting utensils, and should be used as hot as possible.

The rules which have been given will suffice in most cases, and we believe therefore that we may limit ourselves to these general directions. If in special cases modifications are necessary, these will readily suggest themselves.

It is hoped that this little book will help to make known the nature of the commonest diseases of milk, and aid those interested in preventing and in combating them.

THE END.



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